

REMOVAL OF AMMONIA (NITRIFICATION) IN CONVENTIONAL AND MICROBIAL FUEL CELL TYPE BIOREACTORS

A thesis submitted to the
College of Graduate Studies and Research
in partial fulfillment of the requirement for the
degree of Master of Science in the
Department of Chemical and Biological Engineering
University of Saskatchewan
Saskatoon, SK
Canada

By
Uranbileg Daalkhaijav

Permission to use

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis. Requests for permission to copy or to make other uses of materials in this thesis in whole or part should be addressed to:

Head of the Department of Chemical Engineering
University of Saskatchewan
57 Campus Drive
Saskatoon, Saskatchewan S7N 5A9
Canada

OR

Dean
College of Graduate Studies and Research
University of Saskatchewan
107 Administration Place
Saskatoon, Saskatchewan S7N 5A2
Canada

Abstract

The challenge of access to clean water is apparent throughout the world as it experiences overpopulation, the resulting pollution, and struggle with various manifestations of global climate change. In addition, as the first world countries are able to enjoy clean drinking water, they are increasing the global energy demand thereby exacerbating the pollution of limited water resources and global climate change. Nitrogenous compounds such as ammonia, nitrate and nitrite are among the major water pollutants. In this work, biological removal of ammonia and nitrite from contaminated waters and potential for producing electricity were studied using conventional bioreactors and microbial fuel cell (MFC) type bioreactors. Performance of the microbial fuel cell (MFC) has been compared with bioreactors which are conventionally used for nitrification in wastewater treatment plants. Specifically, effects of the contaminant concentration and the types of nitrogenous contaminants (ammonia and nitrite) on the removal rate of ammonia and nitrite, as well as generation of electricity have been investigated.

Findings of the present work in the conventional reactors with free cells and biofilms revealed that oxidation of ammonia to nitrite and produced nitrite to nitrate takes place sequentially. The removal rates of both ammonia and nitrite are directly related to feed concentration up to 60 mM. The biofilm reactor was able to handle much higher loading rates of contaminants and led to much higher removal rates at shorter residence times when compared with the continuous reactor (CSTR) with free cells. This was probably due to higher biomass concentration in the biofilm system. The continuous operation of CSTR and biofilm reactors demonstrated that removal rates of both ammonia and nitrite were dependent on the reactor loading rates and that loading rate (residence time) can be used to control the composition of end products during the nitrification process. Finally, results obtained in the microbial fuel cell bioreactors have shown that efficient removal of ammonia and nitrite (nitrification)

and generation of electricity can be successfully achieved in this system. Nitrite removal rates obtained in MFC without a mediator are comparable to that obtained in the conventional reactor for certain concentration ranges. While with ammonia, comparable rates are only achieved in the presence of mediator (resazurin).

Acknowledgements

Studying at University of Saskatchewan and being a part of the community has been a great opportunity for me. I was fortunate to be able to work under the guidance of Dr. Mehdi Nemati for the past two years. I would like to thank my supervisor Dr. Nemati for all his patience, good judgment, and his willingness to share from his wealth of knowledge. In addition, I would like to thank the members of my committee Dr. Richard Evitts and Dr. Jafar Soltan for their input and time. The faculty at University of Saskatchewan has also been great source of knowledge for me, including the people who basically acted as the support team for our lab such as Richard Blondin, Dragan Cekic, and Heli Eunike. This work was made possible through the generous support of the Agriculture Development Fund from the Ministry of Agriculture, Saskatchewan and a Discovery Grants from the Natural Sciences and Engineering Research Council of Canada (NSERC).

My parents, Enkhee and Daalkhai, are such awesome people and I want to thank them for all that they have done for me. So mom and dad, thank you again for putting up with me for so many years and letting me live with you and eat all your food. I promise, when you get old and don't want to do your own groceries, you are welcome to come live with me. I want to thank my little brother Amraa for his moral support and let him know that I'm glad I didn't succeed in trading him for a box of apples when he was one year old.

Dedication

This work is primarily dedicated to my grandmother

Devee,

who is one of the strongest, shrewdest, and proudest women I know,

and my late grandmother

Dolgor

who took care of me when I was sick and taught me to read.

Table of Contents

List of Figures	VIII
List of Tables	IX
1 INTRODUCTION	1
1.1 World's Water.....	1
1.2 Sources and Dangers of Contaminants in Bodies of Water	1
1.3 Ammonia as a Contaminant	2
1.4 Research Direction	2
2 LITERATURE REVIEW	4
2.1 Conventional Nitrification and Denitrification Processes	4
2.2 Alternatives to Conventional Nitrification and Denitrification	6
2.2.1 Simultaneous Nitrification and Denitrification (SND).....	7
2.2.2 Partial Nitrification (Shortcut Nitrification and Denitrification).....	8
2.2.3 ANAMMOX.....	14
2.2.4 Summary of biological nitrogen removal methods	18
2.3 Microbial fuel cell technology.....	19
2.3.1 Cathodic catalysts.....	20
2.3.2 Reactor configurations	23
2.3.3 Treatment of waste streams using microbial fuel cells	26
3 RESEARCH OBJECTIVES	32
3.1 Knowledge Gap	32
3.2 Objectives	32
4 MATERIALS AND METHODS	33
4.1 Medium	33
4.2 Microbial Culture	34
4.3 Experimental setup	35
4.3.1 Batch trials	36
4.3.2 Continuous trials: CSTR and biofilm reactors.....	38
4.3.3 Dual chamber microbial fuel cell trials	40
4.4 Analyses.....	45
4.4.1 Measuring ammonia.....	45
4.4.2 Measuring nitrite and nitrate.....	46

4.4.3	Measuring protein	46
5	RESULTS AND ANALYSIS	48
5.1	Ammonia removal in batch systems	48
5.1.1	Batch reactor with free cells	48
5.1.2	Batch reactor with biofilm	50
5.2	Nitrite removal in batch systems	52
5.2.1	Nitrite oxidation batch reactors with free cell	52
5.3	Rate of oxidation in batch system	54
5.3.1	Ammonia oxidation rate	54
5.3.2	Nitrite oxidation rate	56
5.3.3	Comparison of ammonia and nitrite oxidation rate	58
5.4	Ammonia removal in continuous systems	59
5.4.1	Ammonia removal rate as a function of loading rate	60
5.4.2	Effluent composition in CSTR	62
5.4.3	Effluent composition in the biofilm reactor	66
5.5	Ammonia removal in batch MFC	70
5.5.1	Batch runs without mediator	70
5.5.2	Batch runs with mediator	73
5.6	Nitrite removal in batch MFC	76
5.7	Power density and current density preliminary analysis	80
6	SUMMARY OF FINDINGS	81
6.1	Recommendation for future work	82
	Bibliography	84
	Appendix A	88
	Standard Curves	88

List of Figures

Figure 2.1 Traditional nitrification and denitrification processes.....	4
Figure 2.2 Redox diagram of nitrification and denitrification (Ahn, 2006).	5
Figure 2.3 Microbial floc - oxygen diffusion limitation creates aerobic zone supporting nitrifiers and anoxic zone supporting denitrifiers.....	7
Figure 2.4 Nitrification and denitrification by nitrite pathway, bypassing nitrite oxidation and reduction steps.....	8
Figure 2.5 Electron rich substrate in the anode (A) is oxidized by the bacteria (B), bacteria gives off the electrons to the anode under anaerobic condition (C), electrons travel through the external circuit (D) to the cathode (F) where the final electron acceptor is either chemical catholyte (G), oxygen being converted to water (H), or is given off to cathode bacteria which goes on to reduce another compound (I). The protons created in the anode as a result of substrate oxidation (A) diffuses through the proton exchange membrane between the anode and the cathode (E), in the cathode it might be involved in the reduction process such as the synthesis of water (H).	19
Figure 2.6 Dual chamber MFC with oxygen as final electron acceptor.....	24
Figure 2.7 Single chamber MFC with oxygen diffusive cathode.....	25
Figure 4.1 Three phases of the research: batch, continuous, and MFC along with each parameter studied during each phase.....	35
Figure 4.2 Free cell culture.....	37
Figure 4.3 Biofilm reactor.	38
Figure 4.4 Continuous reactor set up; biofilm (right), CSTR reactor (left)	39
Figure 4.5 Dual chamber microbial fuel cell - data logger, anode, proton exchange membrane (PEM), cathode, and multimeter (left to right).	41
Figure 4.6 Mediator dye resazurin is deep blue in its oxidized state but turns into pink resorufin form due to reduction (O'Brien et al., 2000).....	43
Figure 5.1 Concentration of various ions during oxidation of 5 (A), 15 (B), 36 (C), and 73 (D) mM initial ammonia concentration in free cell batch reactors. Data represent the average value of concentrations from duplicate experiments and error bars are the associated standard errors.....	49
Figure 5.2 Concentration of various ions during oxidation of 3 (A), 15 (B), 36 (C), 100 (D) mM ammonia in biofilm batch reactors. Data represent the average value of concentrations from duplicate experiments and error bars are the associated standard errors.	51
Figure 5.3 Concentration of various ions during oxidation of 6 (A), 15 (B), 30 (C), and 60 (D) mM nitrite in batch reactors with free cells. Data represent the average value of concentrations from duplicate experiments and error bars are the associated standard errors.	53
Figure 5.4 Ammonia oxidation rate for A) biofilm and B) free cell reactors. Vertical error bar represents the standard error in the calculated oxidation rate and the horizontal bar represents the standard error in the measured initial concentration for duplicate biofilm and free cell trials.....	55
Figure 5.5 Nitrite oxidation as a function of initial nitrite concentration – A) biofilm reactor, starting substrate ammonia, B) free cell reactor, starting substrate ammonia, and C) free cell reactor, starting substrate nitrite. Vertical error bar represents the standard error in the oxidation rate and the horizontal bar is the standard error in initial concentration for duplicate trials.	57
Figure 5.6 Average ammonia and nitrite oxidation data as a function of initial nitrogen concentration. In the parenthesis, the first variable denotes the type of starting substrate in the batch (sodium nitrite or ammonia) and the second variable denotes the type of culture used (FC- free cell, BF- biofilm). The regression lines in blue are for oxidation of nitrite and in orange are for oxidation of ammonia.....	58
Figure 5.7 Transient data in biofilm reactor operated with 17 mM ammonia at feed rate of 10 mL/h.	60
Figure 5.8 Ammonia removal rate as a function of its loading rate for A) CSTR and B) biofilm reactors.	61

Figure 5.9 Effluent composition of CSTR, A) ammonia, B) nitrite and C) nitrate, at different feed ammonia concentration as a function of loading rate up to 8 mM/h.....	63
Figure 5.10 Composition of CSTR effluent nitrogen for feeds with A) 17.6 mM, B) 31.5 mM and C) 63.2 mM ammonia.....	65
Figure 5.11 Effluent composition of biofilm, A) ammonia, B) nitrite and C) nitrate, at different feed ammonia concentration as a function of feed loading rate.	67
Figure 5.12 Composition of biofilm reactor effluent for A) 17.6 mM, B) 31.8 mM and C) 61.5 mM ammonia concentration.....	69
Figure 5.13 MFC ammonia oxidation with A) completed 18.1 mM, B) incomplete 18 mM, and C) incomplete 30.6 mM ammonia batch results.	72
Figure 5.14 First ammonia oxidation batch with resazurin as electron mediator.	73
Figure 5.15 Ammonia oxidation in MFC with resazurin mediator containing A) 8 mM , B) 11 mM, and C) 24 mM ammonia.....	75
Figure 5.16 Sequential oxidation of nitrite: A) first batch, B) second batch, and C) third batch.	77
Figure 5.17 The nitrite oxidation batches without mediator at A) 8 mM, B) 18 mM, C) 35 mM, and D) 60 mM concentrations	79

List of Tables

Table 2.1 Nitrite pathway control parameters (Paredes et al., 2007)	9
Table 2.2 Comparison of biological nitrogen removal technologies (Zhu et al., 2008).....	18
Table 4.1 Composition of medium	33
Table 4.2 Composition of trace elements.....	33
Table 4.3 Summary of all the trials and their parameters.	36
Table 5.1 Current and power density data for ammonia and nitrite oxidation reactions in MFC.....	80

1 INTRODUCTION

1.1 World's Water

Global demand for fresh water is drastically rising and the supply is becoming increasingly scarce since the start of the industrial era. Science and technology have brought the first world countries clean water but it also resulted in increased personal water usage and many developing countries are catching up due to modernization. Personal usage of this resource is completely overshadowed by the heavy industrial and agrarian use. According to a report by UNICEF (World Water Assessment Programme & UNESCO, 2003), only 8% of the fresh water being utilized in the world is for domestic use, while 22% is used by the industrial sector and 70% is used for irrigation. From the 2006 estimates by the Pacific Institute (Gleick et al., 2009), the world annually uses 3714 km³ of fresh water with a projected increase of about 40% by 2025 (World Water Assessment Programme & UNESCO, 2003). The same UN report estimated that the 1,500 km³ of wastewater being produced per year pollutes 8 times its volume of freshwater, resulting in 12,000 km³ of polluted water (World Water Assessment Programme & UNESCO, 2003). This puts an immense pressure on the already dwindling clean water reserves in the world and makes it that much more important to properly treat the wastewaters and runoff from these industrial and agrarian sites in order to meet the present and future demands for this precious commodity.

1.2 Sources and Dangers of Contaminants in Bodies of Water

One of the main sources of river, lake, and groundwater pollution is from agricultural activities such as runoff from fertilizer treated lands and seepages from lagoons holding industrial farm animal waste (GRACE Communications Foundation, 2011). These types of non-point source pollutions are harder to treat and are often overlooked compared to the point source pollutions. Environmental Protection Agency (EPA) report in 2000 accessing America's rivers and streams showed that 39% of the

rivers surveyed were contaminated. Agriculture was the source of the contaminants for 48%, making it the leading contaminator for river systems (U.S. Environmental Protection Agency, 2000). In the same report, it was shown that 22% of lakes in United States had high level of nutrient contamination (nitrogen and phosphorus) and this was the most common type of pollutant in all the lakes. This type of contamination causes what is known as eutrophication. Eutrophication is the drastic proliferation of algae due to high level of nutrients that causes the body of water to appear blue-green but more importantly, the subsequent death and decomposition of the algae by microorganisms use up much of the dissolved oxygen resulting in the destruction of the aquatic life (Art, 1993).

1.3 Ammonia as a Contaminant

Ammonia is used in large amounts in certain industries such as plastics, synthetic fibers, explosives, condensation catalyst, neutralizing agent, pharmaceutical, preservatives, metal treating, petroleum, industrial refrigeration, and is a key ingredient in all the fertilizers (Milne, 2005). Furthermore, ammonia is a byproduct of activated sludge process which is used to remove organic compounds (BOD) in wastewater treatment facilities. From all the different methods to treat ammonia, biological nitrification and denitrification is the most common method and is used in the majority of the treatment plants due to its simplicity and feasibility (Ahn, 2006). In biological nitrification, ammonia is aerobically converted by bacteria to nitrate, while anaerobic denitrifiers reduce nitrate to nitrogen gas in the presence of a carbon source.

1.4 Research Direction

This research is aimed at studying the nitrification step of the ammonia treatment process in conventional reactors as well as in the microbial fuel cell type bioreactors so that energy can be created

at the same time the waste is being treated. This would help determine the feasibility of using the nitrification process to generate power as well as allow for a performance comparison between conventional and microbial fuel cell reactors as far as ammonia treatment is concerned.

This thesis contains six chapters including this introductory chapter. The second chapter contains a literature review of topics on conventional as well as alternative nitrification and denitrification methods and microbial fuel cell technology. The third chapter defines the research objectives while the fourth chapter lays out the experimental setup of the three phases of this research. Results for the batch, continuous and microbial fuel cell phase of the research are found in chapter five. Chapter six contains concluding remarks and plans for the future.

2 LITERATURE REVIEW

2.1 Conventional Nitrification and Denitrification Processes

Currently many wastewater treatment facilities handle ammonia through biological nitrification and denitrification treatment that converts ammonia to nitrogen gas as shown in Figure 2.1.

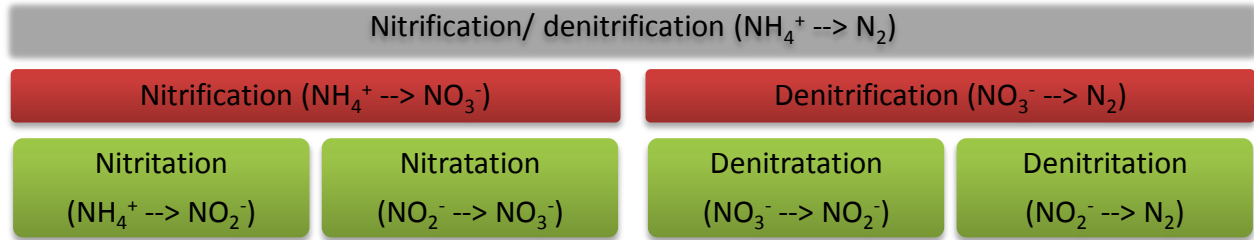
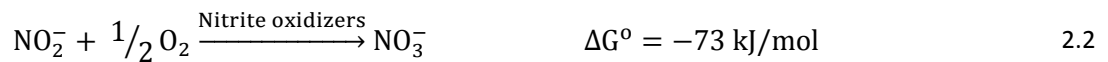
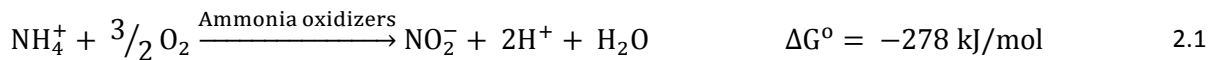
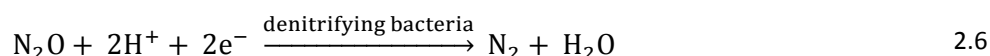
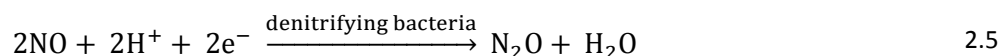
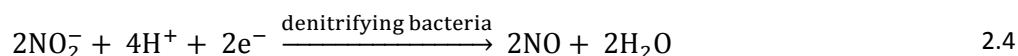
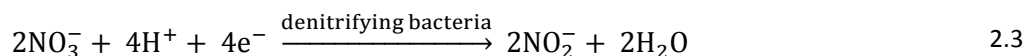


Figure 2.1 Traditional nitrification and denitrification processes.

The first step of the treatment process is the nitrification. It takes place in an aerated basin containing nitrifying bacteria and inorganic carbon source. Each oxidation step is a distinct process involving separate groups of bacteria. The ammonia oxidizing bacteria (AOB) convert ammonia to nitrite, and then the nitrite oxidizing bacteria (NOB) convert nitrite to nitrate. The process of ammonium oxidation to nitrite is called nitrification and can be described by equation 2.1. Further oxidation of nitrite to nitrate is referred to as nitrification and proceeds according to equation 2.2 (Cuidad et al., 2005).



The most common types of AOBs found in wastewater treatment plants (WWTP) are *Nitrosomonas europaea/ eutropha*, *Nitrosomonas oligotropha*, *Nitrosomonas communis*, and *Nitrospira* lineages, while the common NOBs include *Nitrobacter* and *Nitrospira* species (Siripong & Rittmann, 2007). The second stage of ammonia treatment is denitrification. Denitrification takes place in an anaerobic or oxygen limited basin. This step reduces nitrate back to nitrite which is further converted to nitrogen gas and released into the atmosphere according to equations 2.3, 2.4, 2.5, and 2.6 (Clauwaert et al., 2007).



One of the hazards of contaminants such as ammonia in water is that it can convert into nitrates and can easily seep into shallow groundwater such as private wells (Nolan et al., 2002). Also, free ammonia or un-ionized NH_3 is extremely toxic to aquatic life (Carrera et al., 2003). It is necessary to further treat nitrate because nitrate is a health hazard to humans. According to the World Health Organization (2011), nitrate can be reduced to nitrite by the bacteria in the gastrointestinal tracks by oxidizing iron in the hemoglobin, therefore reducing the blood hemoglobin's affinity to oxygen. While most people would be fine with certain level of oxidized hemoglobin or methemoglobin in their system, vulnerable groups such as infants can be fatally affected, which is the reason the common term for this condition is the "blue baby syndrome" (World Health Organization, 2011).

The nitrification steps raise the oxidation state from -3 to +5 and the denitrification steps lower the oxidation state down to zero (Ahn, 2006). The entire process results in the net loss of 3 electrons by nitrogen as shown in Figure 2.2.

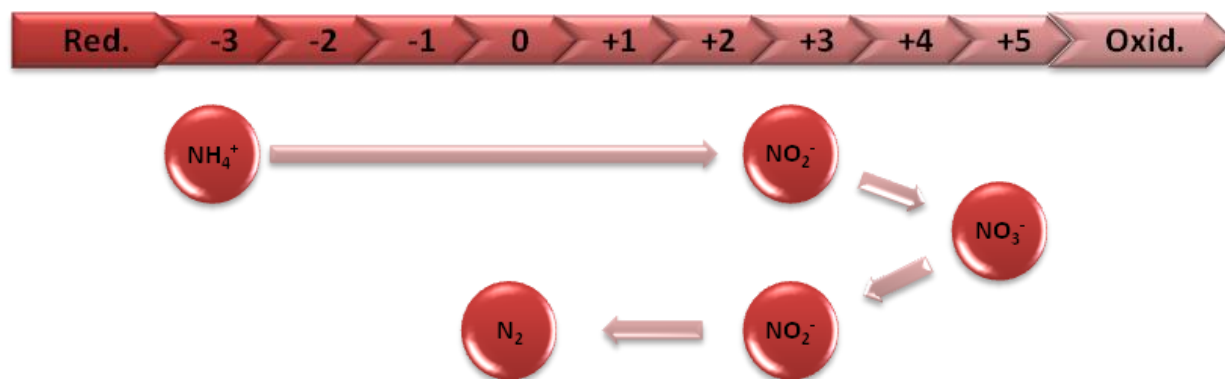


Figure 2.2 Redox diagram of nitrification and denitrification (Ahn, 2006).

These characteristics of the nitrification reactions offer the potential for this to be carried out effectively in the microbial fuel cell technology. The nitrification step yields excess electrons for the bioanode but the final product of nitrification, nitrate, is a substance with one of the highest redox potentials after oxygen and iron (III) (He & Angenent, 2006). The potential of oxygen as electron acceptor is 0.82 V, iron (III) is 0.77 V, ferricyanide is 0.36 V, and nitrate is 0.74 V against standard hydrogen electrode (He & Angenent, 2006). This high potential of nitrate makes it an ideal alternate electron acceptor in a biocathode. Nitrate can replace the expense of constant air pumping to supplement oxygen and eliminates the problem of disposal in respect to using toxic ferricyanide as the final electron acceptor. The oxidation potential of nitrification can drive the anode while the reduction potential of the product of nitrification functions as a viable electron acceptor in the cathode through the denitrification process.

2.2 Alternatives to Conventional Nitrification and Denitrification

Much of the recent research on nitrification has concentrated on adjusting the traditional process in order to enhance the treatment capability or decrease the energy and costs associated with it. Many of the proposed variations on the conventional nitrification and denitrification processes address the issues of aeration costs and supplemental organic carbon demand. The nitrification process requires large amounts of air and the denitrification process often needs additional organic carbon such as methanol, acetate, and glucose as an energy source and electron donor (Ahn, 2006). Review of biological nitrogen removal technologies by various researchers has identified alternative nitrogen removal processes that are novel and cost effective. These include simultaneous nitrification and denitrification, partial nitrification, anaerobic ammonium oxidation, aerobic deammonitrification, completely autotrophic nitrogen removal over nitrite, and oxygen limited autotrophic nitrification denitrification (Ahn, 2006; Parades et al., 2007; and Zhu et al., 2008).

2.2.1 Simultaneous Nitrification and Denitrification (SND)

The conventional nitrification and denitrification process needs to be operated in separate vessels sequentially. However, through simultaneous nitrification and denitrification (SND), the two distinct processes can be unified. The mechanism of SND can be either physical or biological. The physical mechanism takes advantage of the natural oxygen concentration gradient that form inside the activated sludge flocks (Figure 2.3). The outer region of the flock would be exposed to higher level of oxygen so it supports nitrification reaction and the center of the flock, the region exposed to less than 0.5 mg DO/L, support denitrification reaction (Zhu et al., 2008).

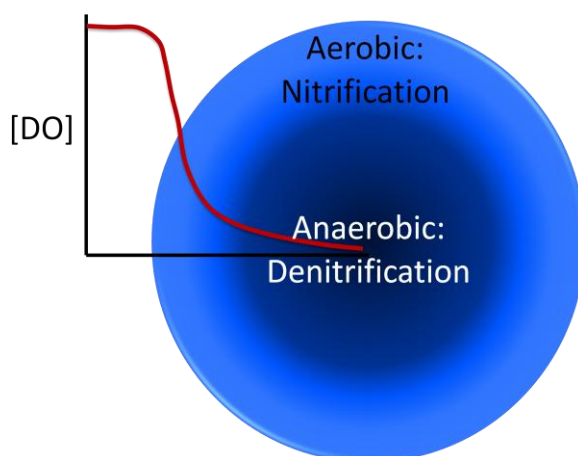


Figure 2.3 Microbial floc - oxygen diffusion limitation creates aerobic zone supporting nitrifiers and anoxic zone supporting denitrifiers.

The biological mechanism utilizes heterotrophic nitrifiers such as *Alcaligenes sp.*, *Corynebacterium sp.*, *Acinetobacter sp.*, *Xanthomonas sp.*, *Bacillus sp.* and aerobic denitrifiers such as *Paracoccus denitrificans*, *Microvirgula aerodenitrificans*, and *Thaurea mechernichensis* (Zhu et al., 2008). The SND process has the benefit of integrating the nitrogen removal processes and saving the costs of an extra reactor.

The influential parameters controlling SND process are the carbon and DO concentrations, and the floc size. There needs to be adequate amount of organic carbon for the complete denitrification. The BOD level which is typically present in wastewater (100-150 mg/L) is sufficient for this purpose (Zhu et al., 2008). The DO concentration needs to be low enough to provide the anoxic condition that enables

both nitrification on the surface and denitrification inside the floc. The size of the floc needs to be larger than 125 μm in diameter in order to provide the aerobic and anaerobic zones within the particle (Zhu et al., 2008).

2.2.2 Partial Nitrification (Shortcut Nitrification and Denitrification)

The other alternative to nitrification and denitrification processes is bypassing the nitrite oxidation step through incomplete oxidation of ammonia to nitrite (nitrite pathway). This would accumulate nitrite which is then reduced to nitrogen gas in the denitrification step as shown in Figure 2.4. The main mechanisms used to achieve nitrite accumulation are the AOB promotion and NOB inhibition. For the rest of this chapter, shortcut nitrification and denitrification, partial nitrification, and nitrite pathway will be used interchangeably and refer to the same process. Zhu et al. (2008) highlighted three main benefits of shortcut nitrification and denitrification method:

1. 25% reduction in aeration resulting in 60% reduction in process energy consumption.
2. 40% reduction in the required amount of electron donor (typically organic carbon).
3. Shorter overall residence time as nitrate reduction rate takes 1.5 – 2 times longer than nitrite reduction.

The partial nitrification process is thought to be most economic for low C:N ratio wastewater such as the high strength sludge from municipal wastewater treatment plants.

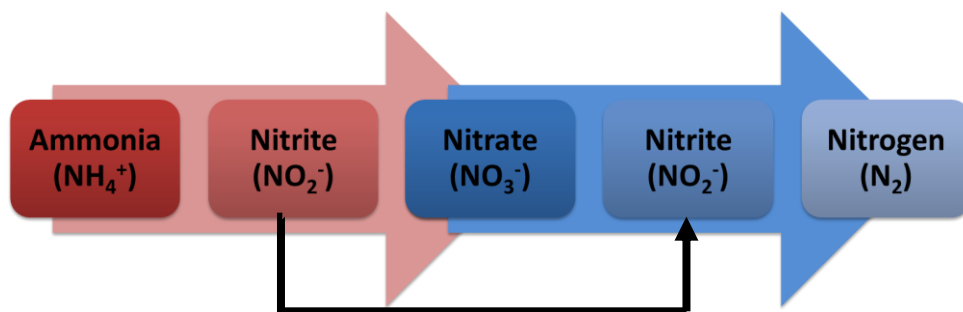


Figure 2.4 Nitrification and denitrification by nitrite pathway, bypassing nitrite oxidation and reduction steps.

2.2.2.1 Control Strategy Enabling Partial Nitrification

There are multiple control parameters that can be used to promote the partial nitrification process. Table 2.1 is a summary of the main parameters and their effects on the nitrification process. The following sections discuss in detail the control factors such as temperature, pH, dissolved oxygen, hydraulic retention time, free ammonia, and free nitrous acid that enable AOB promotion or NOB inhibition.

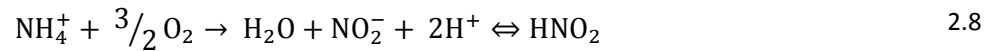
Table 2.1 Nitrite pathway control parameters (Paredes et al., 2007)

Factor	Effect
Temperature	
T > 15 °C	Ammonium oxidizers grow faster than nitrite oxidizers.
pH	
7.0–8.0	Optimum range for nitrification.
7.9–8.2	Optimum range for ammonium oxidizers (<i>Nitrosomonas</i>).
7.2–7.6	Optimum range for nitrite oxidizers (<i>Nitrobacter</i>).
Free NH₃[mg/L]	
150	Inhibition of ammonium and nitrite oxidizers.
1.0–7.0	Inhibition of ammonium oxidizers and nitrite accumulation.
HNO₂ [mg/L]	
> 2.8	Inhibition of ammonium and nitrite oxidizers.
DO [mg/L]	
0.5	Suspended growth
6.0	Inhibition of nitrite oxidation and its accumulation.
6.0	Full nitrification.
DO [mg/L]	
0.5	Completely stirred biofilm reactor.
> 0.5	90% nitrite accumulation and 100% ammonium removal.
> 0.5	Nitrate accumulation.

Inhibition of NOB by free ammonia (FA) and free nitrous acid (FNA):

Nitrite oxidizers have been shown to be inhibited by free ammonia (FA) concentrations of 0.1 – 1.0 mg/L, while ammonia oxidizers are inhibited by FA above 10-150 mg/L. Free nitrous acid (FNA) concentration as low as 0.02 mg N/L has been shown to completely stop *Nitrobacter* biosynthesis (Vadivelu et al., 2005), while FNA concentrations between 0.22 to 2.8 mg/L has been shown to inhibit

both nitrifying groups (Anthonisen et al., 1976). Table 2.1 shows summary information on the control parameters of the partial nitrification pathway. Free ammonia exists in equilibrium with ammonium ion, and free nitrous acid is in equilibrium with nitrite ion as represented by equations 2.7 and 2.8 (Anthonisen et al., 1976), respectively.



This relationship between FA and ammonium ion as well as nitrite ion and nitrous acid can be used to determine the FA and FNA concentrations using equations 2.9 and 2.10 (Anthonisen et al., 1976). According to these equations, pH and temperature play key roles in determining the concentration of these inhibitive substances.

$$\text{FA} = \frac{1.214 * [\text{NH}_4^+ - \text{N}] * 10^{\text{pH}}}{e^{6344/T} * 10^{\text{pH}}} \quad 2.9$$

$$\text{FNA} = \frac{46}{14} \times \frac{[\text{NO}_2^- - \text{N}]}{e^{(-2300/T)} * 10^{\text{pH}}} \quad 2.10$$

FA (free ammonia), FNA (free nitrous acid), $[\text{NH}_4^+ - \text{N}]$ (total ammonia N), $[\text{NO}_2^- - \text{N}]$ (total nitrite N): mg/L;

T (temperature): K (Kelvin)

Nitrite accumulation by temperature control:

The previously mentioned inhibitions were used in a study by Kim and Seo (2006) that successfully selected ammonia oxidizers in a sequencing batch airlift reactor over their 100 day trial period. However, a similar study by Kim et al. (2008) using batch reactors found that free ammonia did not affect nitrite accumulation rate, instead the determining factor was temperature. The specific ammonia oxidation rate increased 5.3 times by an increase of temperature from 10 to 30°C, while nitrite

oxidation rate only increased by a factor of 2.6. The temperature effect on ammonia oxidation rate did not change when the culture was exposed to FA concentration of 90.1 mg N/L. The nitrite accumulation rate increased with temperature, and at 30°C reaching 15.4% above the rates seen under 20°C. At 20°C or higher, AOBs grow faster than NOBs therefore outcompete them.

Anthonisen (1976) used Arrhenius equation to express the dependency of the nitrifying bacteria maximum growth rate on temperature (Equation 2.11). At temperatures below 15°C, NOB growth rate (0.642 d⁻¹) is higher than AOB (0.523 d⁻¹) (Zhu et al., 2008). However, AOB have higher maximum specific growth rate ($\mu_{\max} = 0.801 \text{ d}^{-1}$) than NOB ($\mu_{\max} = 0.788 \text{ d}^{-1}$) at 20°C, which allows the AOB to overtake NOB at higher operating temperatures (Zhu et al., 2008). So temperature (°C) is an effective parameter for AOB promotion and nitrite accumulation for partial nitrification.

$$\mu_{\max}(T) = \mu_{\max}(20^{\circ}\text{C}) * \exp \left[-\frac{E_a(20 - T)}{293R(273 + T)} \right] \quad 2.11$$

μ_{\max} (maximum growth rate): d⁻¹, T (temperature): °C, E_a (activation energy): kJ/mol, R (constant): 8.314 J/mol K

Nitrite accumulation by DO control:

Ammonia and nitrite oxidizers have different levels of affinity to oxygen due to the reaction demand as seen from equations 2.1 and 2.2. The oxygen affinity constant (K_s) for ammonia and nitrite oxidizers are 0.3 and 1.1 mg/L, respectively and the DO concentration required for complete nitrification is thought to be 3.5 mg/L (Wiesmann, 1994). This was used in Ciudad et al. (2005) study where they found that at 1.4 mg DO/L, 95% of the ammonia was oxidized and they were able to accumulate 75% of the oxidized ammonia in form of nitrite in an activated sludge reactor with no adaptation by NOBs to low DO for the duration of 275 days of the trial. Ciudad et al. (2005) reduced the

oxygen mass transfer coefficient (K_La) by 40% in their study. Their results were similar to an earlier study by Ruiz et al. (2003) that showed DO concentration of 0.7 mg/L can be used to convert 98% of the ammonia and accumulate 65% of the converted nitrogen as nitrite.

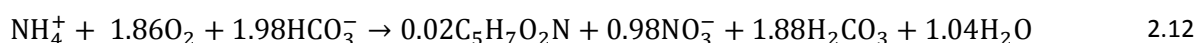
Both Ruiz et al.(2003) and Ciudad et al. (2005) found that DO at a level of 0.5 mg/L significantly inhibit ammonia oxidation and reduce nitrite build up, this is in contrast to Hanaki et al. (1990) study that found DO of 0.5 mg/L can be used to inhibit NOBs without significantly impacting the AOBs. This difference is most likely due to the state of the biological culture, Hanaki et al. (1990) used well mixed suspended cell culture in low ammonia concentration, while Ruiz et al. (2003) and Ciudad et al. (2005) used activated sludge reactor with flocs that cause oxygen mass transfer limitations for the cells. Therefore, DO concentration below 1.5 mg/L enables partial nitrification by inhibiting NOBs without much negative impact on the AOB. This finding was verified in actual treatment centers using full strength municipal wastewater (Zhu et al., 2008). Furthermore, aeration pattern can be employed to enable nitrification through period of aeration followed by denitrification activated by ceasing aeration.

NOB inhibition by sludge age or HRT:

Peng and Zhu (2006) identified sludge age as one of the parameters which could inhibit NOB. Ammonia oxidizers have shorter minimum doubling time (7-8 hours) than nitrite oxidizers (10-13 hours) at higher operating temperatures ($>15^{\circ}\text{C}$), so maintaining a short sludge retention time should wash out the slower growing nitrite oxidizers over time. SHARON reactors are usually operated at sludge retention time (SRT), which in this case is equal to the HRT, below 2 days (Zhu et al., 2008).

NOB inhibition by pH control:

The entire nitrification reaction, including the chemical representation of biomass, is presented by equation 2.12 (Campos et al., 1999). This equation emphasizes the need for proper aeration and bicarbonate supply in order to carry the nitrification process to completion. Bicarbonate not only acts as an alkali to cope with the acidification that occurs during ammonia oxidation, but also serves as the carbon source. So both the alkalinity and the available inorganic carbon need to be regenerated, otherwise ammonia oxidation rate drops (Tijhuis et al., 1995).



Ruiz et al. (2003) showed in an activated sludge reactor that pH range between 6.5 to 8.9 allowed complete oxidation, while pH lower than 6.5 and higher than 8.9 resulted in the complete inhibition of both ammonia and nitrite oxidizers. The basic range is thought to cause increase in free ammonia concentration that inhibit both oxidizers, while the acidic range is thought to increase the nitrous acid concentration, the other nitrification inhibitor.

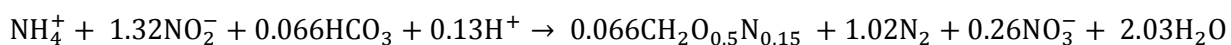
The process pH can be used to affect the FA and FNA concentrations, which in turn can be used to inhibit NOBs. Also, the optimum pH range of AOB is higher (7.9 – 8.2) than NOB (7.2 – 7.6) so slightly more basic reactor condition can be used to favor AOB accumulation, while keeping the FA concentration below 1 mg/L in order to selectively stall nitrite oxidation. The NOBs are sensitive to these compounds at much lower concentrations than AOBs. While an earlier study found nitrite to accumulate at high pH (Suthersand & Ganczarczyk, 1986), Ruiz et al. (2003) showed that it may not be possible to use pH by itself as a method to accumulate nitrite because of adaptation after an initial lag phase. The inhibition zones in terms of pH and concentration can be seen in Anthonisen et al. (1976).

2.2.2.2 SHARON

Single reactor system for high ammonia removal over nitrite (SHARON), utilize the shortcut nitrification and denitrification method. SHARON method is best suited for high ammonia concentrations, >550 mg/L, wastewater (Ahn, 2006). SHARON process utilizes temperature, aeration pattern, and SRT as control parameters to accumulate nitrite and start denitrification in the same reactor. In the SHARON process, both nitrification and denitrification steps occur in the same reactor through aeration control triggering oxidation or reduction, the high temperature favors the AOB, while the short SRT washes out the NOBs. One of the main functions of the denitrification step in the SHARON process is to control the pH during acidification caused by nitrification (Ahn, 2006). In their partial nitrification study with a SHARON reactor, Mosquera-Corral et al. (2005) used the faster growth rate of ammonia oxidizers at 30°C to wash out the nitrite oxidizers with a short sludge retention time (SRT) of 1 day. A full scale SHARON reactor in the Netherlands used sludge digestion effluent as feed with ammonia concentration around 0.5 – 1.5 g N/L and ended up with over 90% conversion to nitrite (van Kempen et al., 2001).

2.2.3 ANAMMOX

The anaerobic ammonium oxidation (ANAMMOX) is carried out by lithoautotrophic bacteria of order *Planctomycetales* (Strous et al., 1998) such as *Procadia anammoxidans*, *Kuenenia stuttgartiensis*, *Candidatus Scalindua brodae*, and *Candidatus Scalindua wagneri* (Zhu et al., 2008). The stoichiometric mass balance of the ANAMMOX reaction, seen in equation 2.13 (Strous et al., 1998), highlights nitrite as an important reactant. In fact, a ratio of 1:1.3 ammonia to nitrite concentration in the influent is needed for optimum reaction. However, a level above 50-150 mg N/L (van der Star et al., 2007) is toxic to ANAMMOX organisms.



Since an inorganic carbon source is used in ANAMMOX, doubling time of bacteria is very long, more than 11 days (Paredes et al., 2007), which has the benefit of reducing the need for sludge treatment. However, it also means that the SRT needs to be kept long to maintain adequate biomass, or retention mechanisms such as biofilm must be used. Dissolved oxygen concentration, temperature, and biofilm thickness are the important factors for this process. The optimum pH for the system is 7.7 – 8.3 and the optimum temperature is between 26 – 28°C (Zhu et al., 2008). Overall, the process could reduce the operating cost by 90% by reducing aeration and eliminating organic carbon.

There are two pathways for ANAMMOX reaction to proceed. First, is to utilize the SND process inside a single reactor. This requires the upper layer of biofilm exposed to higher DO to carry out partial oxidation of ammonia (nitrite accumulation), and the anaerobic zone underneath to carry out the full ANAMMOX reaction. There are multiple variations of the single reactor ANAMMOX process that have been discussed by different research groups. These variations include aerobic deammonification, OLAND, CANON, and denitrifying ammonium oxidation (DEAMOX). One should note that from a biological point of view the processes themselves are similar and the name variations reflect the laboratories in which they were developed.

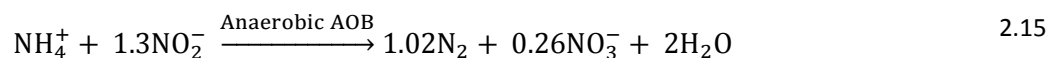
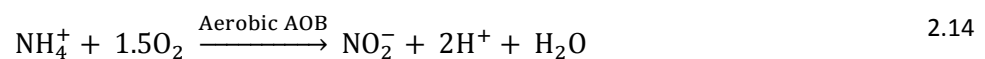
A single reactor system is preferred but it is also much more complex than the two reactor system. In the two reactor system, the first reactor would carry out partial nitrification, converting about 55-60% of the ammonia to nitrite (Ahn, 2006). The second reactor would use the effluent of the first to carry out the ANAMMOX reaction. In the Netherlands, several full scale ANAMMOX WWTP reactors are operational (van der Star et al., 2007). They used reject from sludge dewatering with 750 kg –N/d ammonia loading in a two reactor partial nitrification and ANAMMOX system.

2.2.3.1 Aerobic Deammonification

The aerobic deammonification process is a version of the one reactor ANAMMOX system. The outer layer of biofilm exposed to oxygen carries out nitrification, while the deeper anoxic layer converts ammonia and the intermediate nitrification product such as nitrite, to nitrogen gas in one step (Zhu et al., 2008). Another pathway for this process is for nitrite to be converted to nitrogen gas in the inner layer of the biofilm with NADH_2 as an electron donor (Zhu et al., 2008). This system is best suited for low nitrogen load water such as municipal wastewater. This reaction has been typically observed in conventional nitrification reactors so there has not been a concerted effort to isolate it and optimize it in full scale reactors (Zhu et al., 2008).

2.2.3.2 CANON

The completely autotrophic nitrogen removal over nitrite (CANON) utilizes two types of bacteria, *Nitrosomonas* and ANAMMOX like bacteria (Ahn, 2006), within the same reactor. The main difference between CANON and ANAMMOX process is that in CANON, nitrite acts as an electron acceptor, as opposed to an electron donor in ANAMMOX, so nitrite does not need to be present in the influent wastewater. The entire process occurs in an aerated but oxygen limited environment (DO around 0.5 mg/L) where the oxygen requirements of the first bacteria and the anoxic conditions needed for the second bacteria are met simultaneously. Equations 2.14 and 2.15 demonstrate the two steps in the CANON reaction (Zhu et al., 2008).



The controlling parameters for these reactions are DO, ammonia concentration, and AOB concentration (Zhu et al., 2008). In order to inhibit NOB, either DO or nitrite concentrations need to be kept low. Also, the feed ammonium concentration needs to be around 14 mg/(L*hr) in order to ensure

nearly 100% of the nitrite generated goes through the anaerobic ammonia oxidation step (Zhu et al., 2008). The CANON process reduces aeration by 63%, eliminates the need for organic carbon, and works best for high strength or low C:N ratio wastewaters. The conflicting needs of the CANON process make it very sensitive to changes in DO, temperature, nitrogen loading, and biofilm thickness so further research needs to improve CANON's resistance to shock (Ahn, 2006).

2.2.3.3 OLAND

The oxygen limited autotrophic nitrification – denitrification (OLAND) closely resembles CANON. In OLAND, only aerobic AOB is used, which is slightly different than CANON. Aerobic AOB being the sole biomass makes this process very sensitive to DO concentration, with dramatic AOB inhibition taking place at $\text{DO} < 0.1 \text{ mg/L}$. The main advantage of the OLAND process is its tolerance for fluctuation in ammonium and nitrite concentration better than CANON but it has lower nitrogen removal efficiency (40%) than CANON (Zhu et al., 2008).

2.2.3.4 NO_x

The NO_x process only uses *Nitrosomonas* like bacteria to accomplish nitrification and denitrification in an aerated environment. According to Ahn (2006) this is accomplished by stimulating the bacteria's own denitrifying potential by adding trace amounts of nitrogen oxides (NH_3 to NO_x: 1000 – 5000 to 1). The regulatory capacity of the NO₂ makes the nitrifying culture very sensitive to the levels of nitrite in the reactor.

2.2.4 Summary of biological nitrogen removal methods

Alternative nitrogen removal methods mentioned in this chapter sound promising in reducing aeration, organic carbon, and secondary reactor costs. However, much more research is needed to get these processes fully under control. Table 2.2 summarizes various biological processes used for removal of nitrogen and the important parameters affecting each process.

Table 2.2 Comparison of biological nitrogen removal technologies (Zhu et al., 2008).

	Conventional	SND	SHARON	ANAMMOX	Aerobic deammon.	CANON	OLAND
No. of reactors	2	1	2	1	1	1	1
Feed	WW	WW	WW	$\text{NH}_4^+, \text{NO}_2^-$	WW	WW	WW
Discharge	$\text{NO}_3^-, \text{NO}_2^-, \text{N}_2$	N_2	$\text{NO}_2^-, \text{N}_2$	$\text{NO}_3^-, \text{N}_2$	N_2	$\text{NO}_3^-, \text{N}_2$	N_2
Operating conditions	Aerobic, anoxic	Aerobic	Aerobic, anoxic	Anaerobic	Aerobic	Anoxic	Anoxic
O₂ requirements	High	Low	Low	None	Low	Low	Low
Biomass retention	None	None	None	Yes	Yes	Yes	Yes
BOD requirements	Yes	No	No	No	No	No	No
Sludge production	High	Low	Low	Low	Low	Low	Low
N loading (kg N/(m³* d))	2–8	1–3.5	0.5–1.5	10–20	1–2	2–3	0.1
N removal	95%	100%	90%	87%	60%	75%	85%
Temperature (°C)	12–35	20–30	Above 25	30–40	Unknown	30–40	30–40
Status	Established	Lab	Full-scale	Full-scale	Lab	Lab	Lab
Electron donor	COD	Unknown	COD	NH_4^+	NH_4^+	NO_2^-	NH_4^+

There have been multiple locations around the Netherlands that have successfully implemented alternative nitrogen removal methods in full size wastewater treatment plants. They used the reject water from the sludge digestion process and they treated it through SHARON (van Kempen et al., 2001)

and ANAMMOX (van der Star et al., 2007) processes. SHARON and ANAMMOX processes are most efficient with high strength wastewater. Drawback of SHARON is that it requires high operating temperature, while ANAMMOX requires equimolar nitrite from the feed. The single reactor ANAMMOX system types such as aerobic deammonification, CANON, and OLAND are more complicated therefore prone to system shock and lower nitrogen removal efficiency.

2.3 Microbial fuel cell technology

Microbial fuel cell (MFC) technology has the capacity to transform the biochemical energy from the bacterial conversion of organics and other chemicals to an electrical energy. The bacterial population in the anaerobic anode section of the MFC breaks down the organics and/or inorganic substrates present in wastewater into carbon dioxide, nitrogen compounds, protons, and electrons (Rozendal et al., 2008). In the absence of oxygen, the usual terminal electron acceptor, electrons are transferred to the anodic electrode and from there to the cathode. The energy generating flow of the electrons are seen in Figure 2.5.

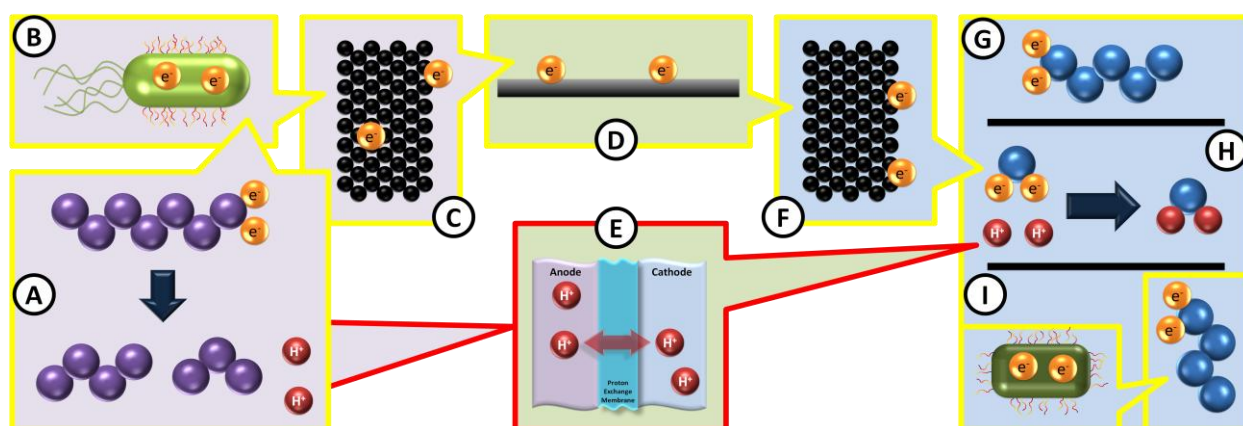


Figure 2.5 Electron rich substrate in the anode (A) is oxidized by the bacteria (B), bacteria gives off the electrons to the anode under anaerobic condition (C), electrons travel through the external circuit (D) to the cathode (F) where the final electron acceptor is either chemical catholyte (G), oxygen being converted to water (H), or is given off to cathode bacteria which goes on to reduce another compound (I). The protons created in the anode as a result of substrate oxidation (A) diffuses through the proton exchange membrane between the anode and the cathode (E), in the cathode it might be involved in the reduction process such as the synthesis of water (H).

The transfer of the intracellular electrons to the electrode could occur through four different mechanisms. The first is through the use of synthetic mediators such as thionine, methyl viologen, neutral red (NR), methylene blue (MB), meldola's blue (MelB), 2-hydroxy-1,4-naphthoquinone (HNO), and Fe(III)EDTA (Du et al., 2007). Second, through naturally produced mediators such as humic acid, anthraquinone, sulphate, and thiosulphate (Du et al., 2007). Either the synthetic mediators penetrate into the cell or the naturally produced mediators within the cell shuttle the electrons from the inside of the bacteria to the surface of the anode (Rabaey & Verstraete, 2005). The third mechanism relies on direct contact between the surface of the electrode and the microorganism which allows redox proteins such as cytochromes to shuttle the electrons to the electrode (Rozendal et al., 2008). And the final mechanism is through microbial appendages referred to as nanowires that transmit the electrons directly from the cell to the electrode (Rozendal et al., 2008). Once the electrons reach the anode, they travel to the positively charged cathode. At the cathode the electrons react with an oxidizing agent such as oxygen or other chemicals in solution that will act as the final electron acceptor.

2.3.1 Cathodic catalysts

While oxygen is a natural oxidizing agent, there is a high cost associated with mechanically aerating the cathodic chamber. Platinum is usually utilized as a cathodic catalyst in order to reach a higher oxygen reduction rate. There is ongoing research on the types of catalysts that could replace platinum in order to keep the cost of operations low. The following sections discuss some of these alternatives.

2.3.1.1 Chemical electron acceptor

One way around the expense of reducing oxygen is to use a soluble chemical oxidizing agent in solution such as potassium ferricyanide. The iron (III) in ferricyanide easily reduces to iron (II) in form of

ferrocyanide, according to equation 2.16 (Oh et al., 2004). However this catalyst is exhausted after a certain time, requiring frequent replacement.



These together with the toxic nature, limit the usefulness of ferricyanide in a large scale and points to the need to develop an electron acceptor that can be easily regenerated.

2.3.1.2 *Platinum*

Platinum has been successfully used in many different fuel cells such as direct formic acid fuel cell (Kim et al., 2005) and polymer electrolysis membrane fuel cell (Rozendal et al., 2008) to speed up the rate of oxygen reduction. The platinum is an excellent catalyst for reduction of oxygen and is the most popular catalyst for this reaction (He & Angenent, 2006). However, most conventional fuel cells operate at a high temperature, between 50 – 100°C, and low pH, providing excess amount of protons for the reaction at the cathode. In the Kim et al. (2005) study, the platinum catalyst potential doubled when the temperature was raised from 25°C to 70°C.

It is necessary for the microbial fuel cell to be run at moderate temperatures and neutral pH values close to those of the wastewaters. So despite the cost of platinum at \$1653 per ounce¹, it results in higher potential losses compared to the biocatalyst in the anode due to this mild operating condition (Rozendal et al., 2008). Platinum catalyst is poisoned by compounds of S, P, As, Zn, Hg, halides, Pb, NH₃, and C₂H₂ (Bartholomew, 2001). Both phosphorus and sulfur containing chemicals, as well as ammonia are common compounds found in wastewater. Hydrogen sulfide, like many other sulfur based substances are considered especially troublesome because they adsorb practically irreversibly to the catalyst surface (Bartholomew, 2001).

¹ Platinum Price. Nov. 25, 2010. In USD. <http://platinumprice.org/>

2.3.1.3 Transition metals

The high cost of platinum catalysts has led some researchers to seek less expensive metals with similar ability to reduce oxygen. Iron and cobalt are transition metals that have been shown in a direct methanol fuel cell study to have high activity for oxygen reduction (Cheng et al., 2006). Using cobalt based catalyst, cobalt tetramethylphenylporphyrin (CoTMPP), Cheng et al. (2006) achieved comparable results with that of platinum. This result was independently replicated by Zhao et al. (2005) whose study showed that iron(II) phthalocyanine and CoTMPP can be effectively used as an MFC catalyst with comparable performance to each other and platinum. The cost of iron and cobalt are \$0.37 and \$37.50 per kg² respectively, much cheaper than platinum.

2.3.1.4 Biocatalysts

The ultimate goal of many scientists working with microbial fuel cells is to establish biocatalyst in both the anode and the cathode chambers. The few studies on MFC biocatalysts can be divided into two groups. The first group is the biocatalysts that function under aerobic condition either through metal oxidation or oxygen production. Each catalytic method goes through specific processes but oxygen is used as the final electron acceptor for all of the methods. In the metal oxidation method, abundant metals such as manganese and iron are first reduced from Mn(IV) or Fe(III) to Mn(II) or Fe(II) abiotically through direct contact with the electrode (He & Angenent, 2006). Then, Mn(II) or Fe(II) are oxidized by the manganese oxidizing bacteria such as *Leptothrix discophora* or iron oxidizing bacteria such as *Thiobacillus ferrooxidans* (He & Angenent, 2006). There was only one study that actually tested the biocatalyst in a complete MFC, the Mn(II) oxidation study by Rhoads et al. (2005), while the rest was

² Metal Prices. Nov. 29, 2010.

http://www.metalprices.com/pubcharts/Public/Platinum_Price_Charts.asp?WeightSelect=KG&SizeSelect=M&ccs=1&cid=0

tested on half cells using external power supply (He & Angenent, 2006). The oxygen production method utilizes blue-green marine algae to convert carbon dioxide and light energy into oxygen. The drawback of this process is the fact that it needs to take place in the light, so if it was scaled up, oxygen production and power generation would only occur during the day (He & Angenent, 2006).

The second group is the anaerobic biocathode group. This method uses other active electron acceptors instead of oxygen, such as nitrate. The redox potential of nitrate is very close to oxygen, $E' = 0.82$ V for oxygen and $E' = 0.74$ V for nitrate versus SHE (He & Angenent, 2006). In various studies using external energy supply, denitrifying bacteria have been shown to effectively reduce nitrate all the way to nitrogen gas in the absence of organic substrate in the media (He & Angenent, 2006). The studies on nitrate reducing biocatalyst has so far been done using external energy source so there is a need for a full microbial fuel cell biocathode study using denitrifying bacteria. While other molecules such as sulfate and carbon dioxide can act as a terminal electron acceptor, their activity is so low that it makes their use impractical.

2.3.2 Reactor configurations

There are several different reactor configuration options for MFC technology which are discussed in the following sections.

2.3.2.1 Dual chamber MFC

The dual chamber MFC configuration, as seen in Figure 2.6, is commonly used in research because of its ease of construction and operation. The separation of the anode and the cathode allows the oxidation and reduction processes to be carried out in independently controlled compartments. In the anode chamber, protons and electrons are produced from oxidation of substrate as a result of

microbial activity. The potential gradient between the anode and the cathode chambers drives the flow of electrons from the anode compartment to the cathode compartment through the external circuit. The concentration gradient drives the diffusion of the protons from the anode to the cathode through the proton exchange membrane. The cathode compartment contains some type of oxidizing agent (either chemical or biological) that uses up the electrons, ensuring the concentration gradient is maintained. In the example in Figure 2.6, oxygen is the final electron acceptor that combines with the hydrogen ions to produce water.

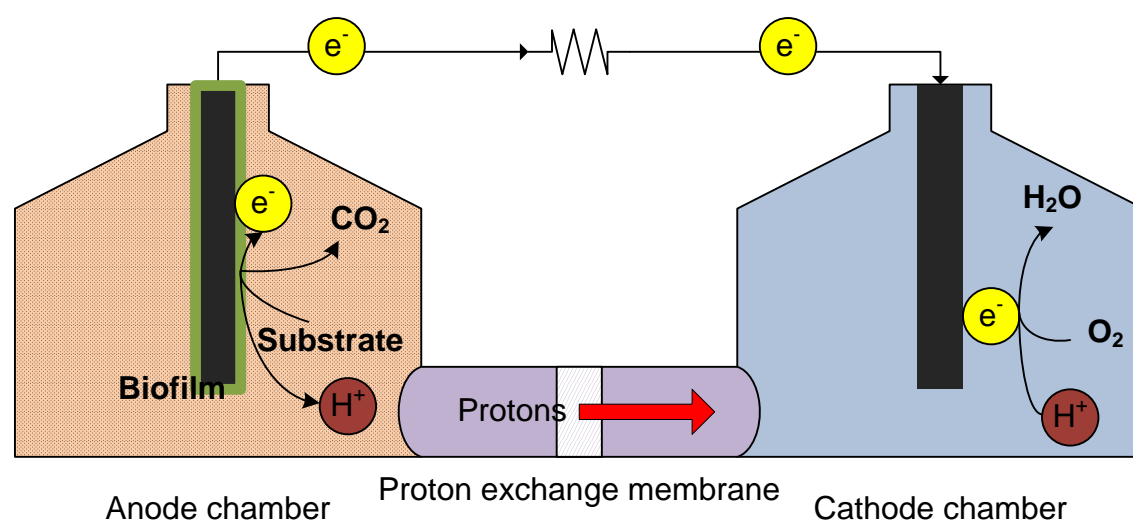


Figure 2.6 Dual chamber MFC with oxygen as final electron acceptor.

2.3.2.2 Single chamber MFC

A typical setup for a single chamber MFC can be seen in Figure 2.7 (Cheng et al., 2006). This system consists of a single chamber containing the microbial culture and substrate where one side of the chamber has the anode electrode oxidizing substrate and the opposite side has the cathode where the final electron acceptor is reduced. The complicated aspect of this set up arises from the fact that anaerobic reaction of the anode side occurs in the proximity of the cathode side that often uses oxygen

as the final electron acceptor. Either an air cathode or an oxygen diffusive cathode would allow both reactions to occur simultaneously.

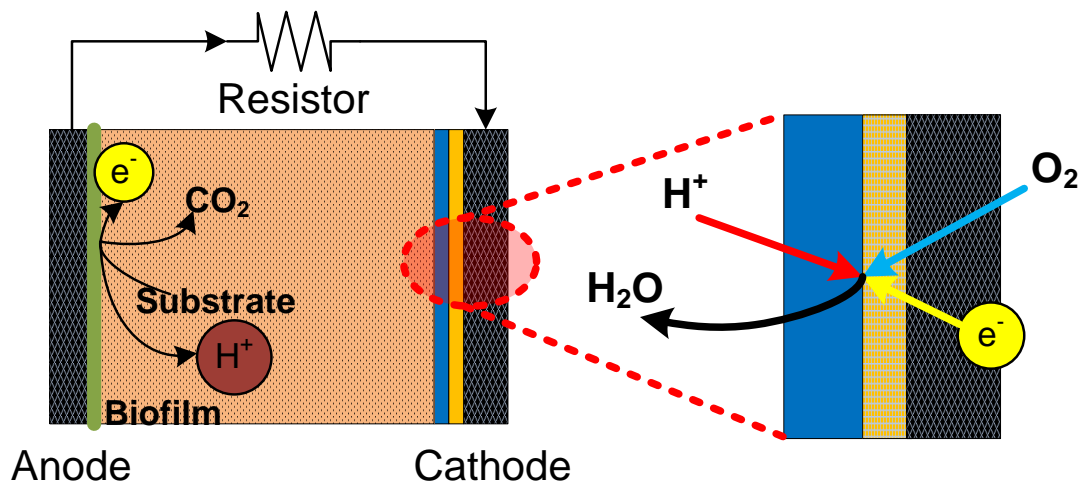


Figure 2.7 Single chamber MFC with oxygen diffusive cathode.

2.3.2.3 Stacked MFC

The stacked configuration utilizes the fact that voltage and current generation in microbial fuel cells can be increased beyond its individual potential by connecting individual cells either in series, providing voltage amplification, or in parallel, providing high current (Liu et al., 2008). Both voltage and current directly affect the power output based on equation 2.17.

$$P = IV = I^2R \quad 2.17$$

P (power): Watts (W); I (current): Amps (A); V (electric potential): Volts (V); R (resistance): Ohms (Ω);

Aelterman et al. (2006) used 6 fuel cells in parallel and series configurations and found that the average power density for the series configuration was 228 W/m³, while parallel configuration was higher at 248 W/m³.

2.3.2.4 Tubular/ Upflow MFC

Tubular or upflow microbial fuel cell design involves feeding the MFC from the bottom of the reactor providing good mixing and sufficient contact time for the reaction of interest to take place before the effluent is discharged at the top. An example of the tubular fuel cell design can be seen in Rabaey et al. (2005) and an example of upflow design can be seen in He et al. (2006). In Rabaey et al. (2005) design, the tubular fuel cell is made of cation permeable membrane filled with granular anode and the cathode is a graphite mat wrapped around the membrane that is soaked with ferricyanide. The He et al. (2005) design concept is similar to the dual chamber model in that the cathode and the anode compartments are separated by PEM. Both anode and cathode chamber was filled with reticulated vitreous carbon, the electron acceptor is oxygen.

2.3.3 Treatment of waste streams using microbial fuel cells

In the last ten years there have been many studies looking at different substrates to use in the microbial fuel cell. Pant et al. (2010) provided an overview of these substrates and the maximum current density obtained using each energy source. Some of the most common substrates that have been used by various researchers include acetate, glucose, lignocellulosic biomass, synthetic wastewater, brewery wastewater, starch processing wastewater, dye wastewater, landfill leachates, cellulose and chitin, and other inorganic substrates (Pant et al., 2010). Simple substrates such as acetate has some of the highest coulombic efficiency (72%) because it is unlikely to go through fermentation or methanogenesis, while complex wastewater substrates such as starch processing water has yielded extremely low efficiency of 7% (Pant et al., 2010). The highest power density mentioned in the Pant et al. (2010) review is 2.15 kW/m³ and 2720 mW/m² both using acetate as substrate in a single chamber MFC. While there is ever increasing number of studies utilizing a variety of organic carbon based substrates as an energy source

for the MFC technology, there has not been many studies looking at one of the most common contaminants in our water systems, nitrogenous products.

2.3.3.1 Ammonia treatment in the microbial fuel cell

The Min et al. (2005) study on electricity generation from swine wastewater reached maximum power density of 45 mW/m² in a dual chamber MFC and 261 mW/m² when they switched to a single chamber MFC. Swine wastewater is characterized by high soluble COD (8320 mg/L), ammonia concentration (198 mg NH₄⁺-N), volatile acids, and orthophosphates (41 mg/L PO₄⁻-P) (Min et al., 2005). Min et al (2005) achieved 88% soluble COD removal and about 83% ammonia removal. They attributed the ammonia removal to nitrification facilitated by oxygen diffusion from the cathode but they did not observe a corresponding increase in nitrite and nitrate. Min et al. (2005) speculate that there might be additional denitrification, anaerobic ammonia oxidation (ANAMMOX), or other ammonia removal processes occurring in the fuel cell.

Kim et al. (2008) took a closer look at the mechanism of ammonia removal in single and dual chamber MFC. The substrate in the anode, swine wastewater, contained 200 ppm ammonia and 1700 ppm COD (Kim et al., 2008). In both types of MFCs, the carbon removal was nearly complete by the end of the experiment. Kim et al. (2008) also found that in a dual chamber MFC, the ammonium removal was nearly 70% and most due to ammonium ions diffusing through the Nafion 117 proton exchange membrane into the cathode to enforce charge balance. For MFC using ferricyanide catholyte, the ammonia loss to the cathode was slower than the oxygen sparged MFC because there was no ammonium removal mechanism in either chambers so once the charge balance was achieved the concentration of ammonia in both of the chambers remained unchanged.

Both single chamber and dual chamber MFCs that use oxygen as the final electron acceptor had almost all their ammonia removal due to stripping of ammonia (Kim et al., 2008). In all the fuel cells, the ammonia stripping increased with electricity generation due to the pH shift around the cathode. Kim et al. (2008) hypothesize that the removal through volatilization mechanism maintains the driving force, ammonium concentration gradient, which makes the oxygen sparge MFC ammonium removal rate faster than the ferricyanide MFC. So while there was over 60% ammonia removal in both types of MFCs, Kim et al. (2008) found that vast majority of the reaction was not biological but rather was the result of physical processes such as diffusion and volatilization.

Lu et al. (2009) used a single chamber air cathode microbial fuel cell to treat starch wastewater which was high in COD (4852 mg/L) and high in ammonia (337 mg/L). The COD removal was 98% while ammonia removal gradually increased from 55.9% to 90.6% with each cycle (Lu et al., 2009). Open circuit ammonia removal was 61.5% while the closed circuit ammonia removal was 90.6% (Lu et al., 2009). There was very little nitrification in Lu et al. (2009) study as there was only small increase in the reactor nitrate concentration (6 mg/L to 16 mg/L) so denitrification, anaerobic ammonia removal, and ammonia volatilization is suspected to be the source of ammonia removal.

Kim et al. (2008) and Min et al. (2005) both used animal wastewater as medium and neither saw any indication that ammonia oxidation which occurred in the MFCs was responsible for the generation of electricity. Due to the presence of organic carbon source, the bacteria are more likely to go through denitrification utilizing the excess electrons in the anode. The anaerobic condition also makes it more likely for alternative nitrogen removal processes such as ANAMMOX to take place. Kim et al. (2008) observed physical removal of ammonia through diffusion and volatilization while Min et al. (2005) reported possible biological removal through nitrification as a result of oxygen diffusion through the cathode and additional denitrification using the organic carbon.

In contrast to these studies, He et al. (2009) found that it is possible to generate electricity using only ammonia as a substrate and bicarbonate as the inorganic carbon source in a rotating cathode MFC. After one day in the MFC with 25 mM of NH_4Cl , nearly 50% of the ammonia was removed where 70-80% of it was nitrite and the rest was nitrate (He et al., 2009). After two months of operation He et al. (2009) were able to achieve a steady current of over 0.06 mA. When the substrate was replaced with either nitrite or nitrate there was no current production. The drawback of this MFC design was that oxygen diffusion into the anode is extremely likely due to the open top container used to house both the anode and the rotating cathode. When the ammonium was used together with either nitrite or nitrate, the current increased from the level of ammonia alone. The nitrite addition to ammonia resulted in a larger increase compared to nitrate (He et al., 2009). Nitrite is thought to be the terminal electron acceptor for ammonia, like in an ANAMMOX process, while nitrate needs to be reduced to nitrite first, resulting in 20% lower current output than nitrite addition to ammonia (He et al., 2009).

You et al. (2009) found that nitrification in the cathode can aid in increasing oxygen reduction rate and cell voltage (0.3 V to 0.567 V) by providing excess protons. Their dual chamber air cathode MFC was fed continuously with synthetic media containing acetate in the anode and ammonium (31 – 94 mg/L) in the cathode (You et al., 2009). The catholyte containing phosphate buffer prevented drastic polarization due to proton depletion in the chamber and was found to be essential in maintaining stable cell voltage (0.525 V) while buffer free catholyte pH quickly shot up to 9 and the voltage dropped below 0.3 V (You et al., 2009). Once ammonium was fed to the cathode, the voltage recovered to 0.567 V, the pH remained steady around 7, and corresponding amount of nitrate was produced without the help of phosphate buffer. The proton supply from 94.2 mg/L ammonium nitrification compensated for proton consumption due to oxygen reduction in the cathode, balanced out the pH increase, and resulted in slightly higher power output (10.94 W/m^3) and cell potential than the 50 mM phosphate buffered catholyte (You et al., 2009).

The above mentioned studies indicate that ammonium can be treated in both the anode and the cathode of a microbial fuel cell. However in the anodic reaction, there are some debate as to whether the ammonium is depleted due to biological oxidation or diffusion/ volatilization and whether it contributes to electricity generation at all.

2.3.3.2 Nitrate treatment in the microbial fuel cell

Park et al. (2005) showed that the product of nitrification, nitrate, can be biologically reduced in the absence of organic carbon in a biofilm electrode reactor using the electrode as the sole electron donor. For nitrate concentrations up to 500 mg/L, they achieved maximum of 98% removal with the application of 200 mA of current (Park et al., 2005). The nitrate was first reduced to nitrite which in turn was reduced to nitrogen gas. Based on Park et al. (2005) findings, Clauwaert et al. (2007) were able to incorporate denitrification into the fuel cell technology. In their dual chamber MFC, the anode was fed acetate solution while the cathode was fed potassium nitrate and bicarbonate as carbon source. After a month of acclimation the fuel cell was able to completely reduce nitrate without nitrite accumulation (Clauwaert et al., 2007). The current production was proportional to denitrification rate where up to 0.146 kg NO_3^-/m^3 was removed at a current of 58 A/ m^3 and the highest power density at 8 W/ m^3 (Clauwaert et al., 2007). Morris et al. (2009) carried out a similar study where denitrification in the cathode resulted in 6.3 mW/ m^2 or 22.2 mW/ m^3 power density using petroleum refinery wastewater in the anode and potassium nitrate in the cathode (Morris et al., 2009). After 22 days the nitrate concentration decreased from 595 to 344 mg/L with no nitrite buildup or at a nitrate removal rate of 11.4 mg/L/day which is lower than the Clauwaert et al. (2007) denitrification rate of 645.5 mg/L/day because of the complexity of the hydrocarbons in the petroleum wastewater (Morris et al., 2009).

Virdis et al. (2008) study looked at the denitrification in the cathode using a slightly different approach. The medium containing acetate and ammonia were fed to the anode where the acetate was oxidized and the effluent was fed to an external reactor where the ammonia was oxidized and that effluent was fed back to the cathode compartment of the fuel cell where the nitrate was reduced (Virdis et al., 2008). This process suffered from several problems such as low coulombic efficiency due to pH polarization and diffusion of ammonia into the cathode through the Ultrex cation exchange membrane. In their next study, Virdis et al. (2010) integrated both nitrification and denitrification processes into their microbial fuel cell and reactor loop system. The anode was fed with acetate and ammonia where the acetate was oxidized and the effluent was fed back to the cathode where the ammonia was removed through simultaneous nitrification and denitrification. Over 94% of nitrogen removal was achieved while producing power of 0.89 mW (Virdis et al., 2010). This process solved the problem that Virdis et al. (2008) had with ammonia diffusion into the cathode and the protons from nitrification prevented pH increase. Both Morris and Jin (2009) and Sukkasem et al. (2008) looked at the impact of nitrate in a single cell microbial fuel cell. In both cases the power of the cell was not affected but the coulombic efficiency decreased. This was due to the competition of nitrate with the anode for the electrons (Morris & Jin, 2009). Sukkasem et al. (2008) achieved 85% removal of 8 mM nitrate and the denitrification rate was not significantly affected by electricity generation (Sukkasem et al., 2008).

Denitrification has been shown to be suitable process for cathodic reduction in the dual chamber microbial fuel cell even though it suffers from coulombic inefficiencies such as pH polarization and the diffusion of ammonia through the cation exchange membrane. While nitrate in the single chamber reactor acted as an alternate electron acceptor thereby reducing the coulombic efficiency. Virdis et al. (2010) showed that a simultaneous nitrification and denitrification process is favorable to denitrification in the cathode.

3 RESEARCH OBJECTIVES

3.1 Knowledge Gap

The processes of nitrification and denitrification have been well studied over the years due to their role as a biological treatment approach for inorganic nitrogenous compounds. Many of the recent studies aim to simplify the nitrification and denitrification steps to a simultaneous process or reduce aeration requirement for nitrification and carbon addition for denitrification. In contrast, MFC technology is relatively new field that is gaining a lot of traction recently due to the global emphasis on “green” energy development. While the concentration and volume of inorganic nitrogen waste can vary drastically according to industry, there is a limited amount of research in the laboratory on the effect of hydraulic retention time and ammonia concentration on the performance of the nitrifying system, especially effects of these variables on the composition of end products. Even less information is available on exploiting the nitrification process in the MFC type reactors for ammonia treatment and electricity generation.

3.2 Objectives

The present research objective is to study ammonia removal through nitrification in the “conventional” bioreactors such as batch and continuous reactors with free-cell and biofilm. The specific effects of ammonia concentration and residence time on the kinetics of nitrification and composition of the end products have been investigated. The other objective was to carry out nitrification in a microbial fuel cell reactor to compare against the conventional reactors and investigating the potential for simultaneous removal of ammonia and generation of energy.

4 MATERIALS AND METHODS

4.1 Medium

A modified form of medium used by Mosquerra-Corral et al. (2005) and trace elements from van de Graaf et al. (1996) were used as the medium throughout this study. Compositions of the medium and the trace element solutions are given in Table 4.1 and Table 4.2, respectively. The inorganic carbon source was sodium bicarbonate. Ammonia in the form of ammonium chloride or nitrite in the form of sodium nitrite was used as the substrate. These were added to the medium in the form of concentrated solutions (3.6 M NH_4Cl -N or 3.6 M NaNO_2 -N) to achieve the desired concentration. The substrate concentrations in the range 4 to 71 mM -N of ammonia and nitrite were tested. Since there is no organic carbon in the medium and since mixed culture was used, it was not sterilized.

Table 4.1 Composition of medium

Compound	Concentration (g/L)
NaHCO_3	3.25
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.30
KH_2PO_4	0.07
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.02
H_2SO_4	0.005
Trace elements 1	1.0 mL
Trace elements 2	1.25 mL
NH_4Cl	0.38 – 3.82
NaNO_2	0.49 – 4.93

Table 4.2 Composition of trace elements

	Compound	Concentration (g/L)
Trace elements 1	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	9.0
	EDTA	6.0
Trace elements 2	EDTA	15.0
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.43
	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.24
	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.99
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.25
	$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.22
	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.19
	H_3BO_3	0.014

4.2 Microbial Culture

The starting culture for this study was a commercial nitrifying culture called Nutrafin Cycle supplied by Hagen³ (Nutrafin Cycle – Biological Aquarium Supplement, Rolf C. Hagen Inc., Canada). This product is normally used in aquariums to treat the accumulated ammonia and nitrite. According to the manufacturer, Nutrafin Cycle contains a flocculated mixture of nitrifying bacteria in solution. While the manufacturer does not specifically identify these bacteria, Nowak et al. (2008) was able to isolate and identify *Nitrosomonas europaea*, *Sporocytophaga*, *Acidobacterium*, *Leifsonia*, and *Clostridiales* species in Nutrafin Cycle through gel electrophoresis. This original culture was adapted to the medium through repeated subculturing in shake flasks. The developed culture was then used in the experiments in CSTR, biofilm, and MFC reactors.

The subculture was made up of 90 mL medium with different levels of ammonia and nitrite as substrate and 10 mL of the previous trial culture as the inoculums. Each batch was made up of this 9:1 mix of new medium to old inoculum. The subculturing took place after all the substrate in the form of ammonia and or nitrite is depleted completely in the reactor. The frequency of the subculturing depended on the batch substrate concentration since higher concentration took longer time to oxidize. The ammonia batches took two to over three weeks to complete while nitrite batches took under a week to 10 days to complete depending on the starting substrate concentration. Throughout the continuous and the MFC phase of the study, batch cultures in 18 mM ammonia and nitrite were maintained at all times on the shaker. These batches were subcultured every two weeks for ammonia and every week for nitrite oxidation and they were maintained in order to use them as inoculums during the continuous and MFC trials.

³ http://www.hagen.com/uk/aquatic/nutrafin/nutrafin_water_conditioning.cfm

4.3 Experimental setup

There were three phases to this research. The phases of the research and the parameters that were specifically studied are presented in Figure 4.1.

1. Conventional batch reactors (free cells and biofilm): i) nitrification of different substrate (ammonia and nitrite), and ii) effect of concentration of substrate.
2. Conventional continuous reactors (free cells and biofilm): the effect of ammonia loading rate controlled by i) flowrate and ii) feed concentration.
3. Microbial fuel cell operated under batch condition using free cell culture: i) ammonia concentration and ii) nitrite concentration.

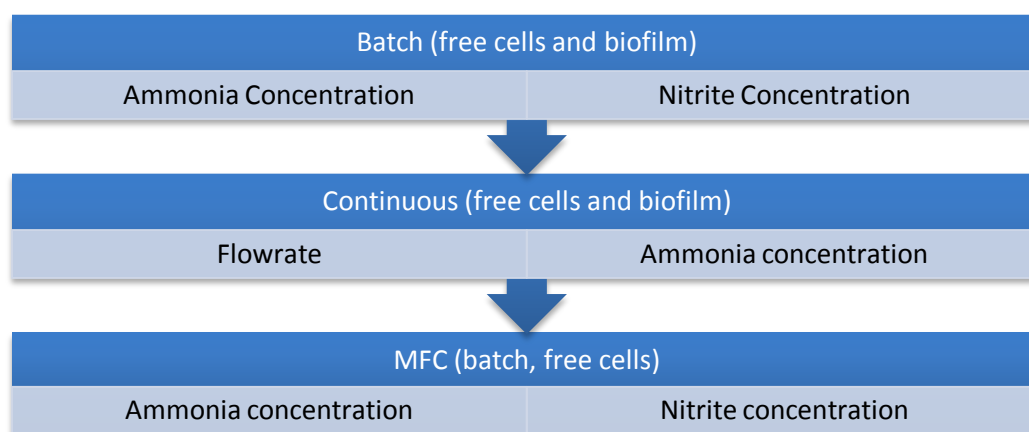


Figure 4.1 Three phases of the research: batch, continuous, and MFC along with each parameter studied during each phase.

Table 4.3 contains the summary information of all the trials conducted during this research along with the parameters of each trial such as type of substrate, investigated factor, state of bacteria, and the range of tested parameter.

Table 4.3 Summary of all the trials and their parameters.

Phase	Substrate	Control factor	Bacteria	Levels
Batch	Ammonia	Concentration	Free-cell	7, 18, 36, 71 mM
Batch	Ammonia	Concentration	Biofilm	4, 18, 36, 71 mM
Batch	Nitrite	Concentration	Free-cell	4, 7, 18, 36, 71 mM
Continuous	Ammonia	Flowrate	CSTR	3-115 mL/hr
Continuous	Ammonia	Flowrate	Biofilm	3-315 mL/hr
Continuous	Ammonia	Concentration	CSTR	18, 36, 71 mM
Continuous	Ammonia	Concentration	Biofilm	18, 36, 71 mM
MFC	Ammonia	Concentration	Free-cell	8, 18, 36 mM
MFC	Nitrite	Concentration	Free-cell	8, 15, 36 mM

4.3.1 Batch trials

4.3.1.1 Free cell batch reactor

The batch reactors were 250 mL Erlenmeyer flasks (Figure 4.2) containing 100 mL of medium with the designated concentration of ammonia or nitrite. The medium was inoculated with the nitrifying stock culture. The reactors were mixed and aerated (surface aeration) using a magnetic stirrer. The experiments were conducted at room temperature (25°C).

The substrate concentrations between 4 to 71 mM ammonia and nitrite were tested. The pH was measured frequently and adjusted using sodium bicarbonate when the substrate concentration was higher than 18 mM NH_3 –N or if the batch was operated in sequence. Two factors conspire to acidify the reactor, first is the oxidation of ammonium which releases protons and second is the consumption of bicarbonate buffer by the bacteria as the carbon source. The target pH range for all the reactors was 7.5 – 8.5 which represents the optimum range for the microbial activity. The acidic condition was treated with 1 M NaHCO_3 where bicarbonate served to replenish the inorganic carbon source and restore the

slightly basic level. The pH is measured using the Orion digital pH probe (PerpHect LogR meter model 330, ThermoFisher Scientific, USA). Reactors were sampled on a regular basis. Concentrations of ammonia, nitrite and nitrate were determined in these samples. Experiments were carried out in duplicate.



Figure 4.2 Free cell culture.

4.3.1.2 Biofilm batch reactor

The biofilm reactor setup can be seen in Figure 4.3. The ceramic rings (Bio MAX biological filter media, Rolf C. Hagen Inc., Canada) made up of porous material designed to allow bacterial colonies to form and thrive in its microstructures, were used as a matrix for the establishment of biofilm. The specific surface area provided by the rings is $9.208 \text{ m}^2/\text{g}$. The biofilm was developed on the ceramic surface over a series of batch trials. For these reactors, the ceramic rings were drained of the spent medium after a batch was complete and replaced with 100 mL of fresh medium. After the nitrifying bacteria built up on the surface of the ceramics during each batch, they were transferred to the next batch when the medium was replaced. Since the ceramics took up about 50 mL volume, the total batch volume was about 150 mL. The medium contained 4, 18, 36, and 71 mM ammonia as substrate. The

reactor was maintained at room temperature ($\sim 25^{\circ}\text{C}$) on a shaker which provided passive aeration. The pH was monitored and adjusted through the same approach described for free cell batch reactors. Some of the biofilm batch reactor data was obtained from using foam filter as the matrix (Super MicroFilter, Rena, USA). The foam was cut up into 1 cm x 1 cm pieces and in total about 112 cm^2 of filter material was used. However, that area does not represent the total surface area of the filter material as that information is not provided by the manufacturer.



Figure 4.3 Biofilm reactor.

4.3.2 Continuous trials: CSTR and biofilm reactors

Figure 4.4 shows both CSTR and biofilm reactors set up for continuous operation. The continuous stirred tank reactor (CSTR) used for the experiments with free cell was a glass vessel with a working volume of 250 mL with influent line for the feed and an effluent outlet. The reactor was aerated through an air stone ($100\text{ cm}^3/\text{min}$) and was continuously mixed using a magnetic stirrer. The continuous biofilm reactor had a working volume of 200 mL. The ceramic rings used as a matrix for establishment of biofilm took up about 50 mL of space so only 150 mL medium was used. About 75 grams of porous rings went into the biofilm reactor so the total surface area of the biofilm is estimated

to be 691 m^2 . The reactor was aerated through an air stone ($200 \text{ cm}^3/\text{min}$) providing even distribution of oxygen and some mixing of the media through the air bubbles. Both reactors (free cells and biofilm) were operated at room temperature. The medium containing the desired ammonia concentration was prepared and transferred into 1 L PVC bags and fed to the reactors using a peristaltic pump.



Figure 4.4 Continuous reactor set up; biofilm (right), CSTR reactor (left)

4.3.2.1 Effect of volumetric loading rate through changing flowrate

In this set of experiments, the ammonia concentration was kept constant at about 250 ppm for both CSTR and biofilm reactors while the feed flowrate was increased stepwise until deterioration in performance of the bioreactor was observed (ammonia removal less than 20%). In the CSTR, the flowrate was increased incrementally from 1 mL/hr to 115 mL/hr. In the biofilm reactor, the flowrate was incrementally increased from 3 mL/hr to 315 mL/hr. At each flow rate sufficient time was given for establishment of steady state conditions. Due to the high substrate loading rates, the timely and frequent control of pH through addition of sodium bicarbonate was extremely important, especially at flowrates below 30 mL/hr. At rates higher than 30 mL/hr, the excess protons were washed out at

sufficient rate that pH adjustment was no longer necessary. Reactors were sampled on a daily basis and samples were analyzed for ammonia, nitrite, and nitrate concentrations. Protein concentration was determined for both CSTR and biofilm reactors. In case of the biofilm reactor, only the cells suspended in solution were measured, not the culture on the surface of the matrix.

4.3.2.2 Changing ammonia concentration

In this set of experiments, the concentration of the influent ammonia was increased successively from 18 to 36 and then 71 mM. For each of these concentrations, three to five different flowrates were tried, 1 to 115 mL/h in CSTR and 3-315 mL/h for biofilm reactor. The flowrates were picked to ensure that at least one flowrate provides data that shows i) complete ammonia and nitrite oxidation to nitrate and ii) partial ammonia and nitrite oxidation. The pH adjustment was necessary and was carried out on a regular basis using a concentrated solution of sodium bicarbonate. Reactors were sampled on a daily basis and samples were analyzed for ammonia, nitrite, nitrate, and protein concentrations.

4.3.3 Dual chamber microbial fuel cell trials

The dual chamber microbial fuel cell used in this work (Figure 4.5) is a system involving several components. The anodic chamber contained the medium with the designated ammonia or nitrite concentration and nitrifying bacteria acting as a biocatalyst for oxidation of ammonia or nitrite. The cathodic chamber contained 50 mM potassium ferricyanide acting as the electron acceptor and 100 mM monopotassium phosphate acting as buffer. Graphite rods with a 6.15 mm diameter and a height of 102 mm were used as electrodes. Chambers were separated by a proton exchange membrane (Nafion 115, DuPont, USA). The anode and the cathode were connected using chromel wires to a multichannel voltmeter (Integra Series 2700 multimeter, Keithley Instruments Inc., USA) which was connected to a data logger installed on a computer. The voltage was measured and logged every 20 minutes. The

anodic chamber was under anaerobic condition. A needle (gauge 16) was injected into the rubber cap to release the pressure created by the evolved gases. Both chambers were mixed using magnetic stirrers and sampled from a port on the side. The MFC had a working volume of 200-250 mL per chamber and operated at room temperature.

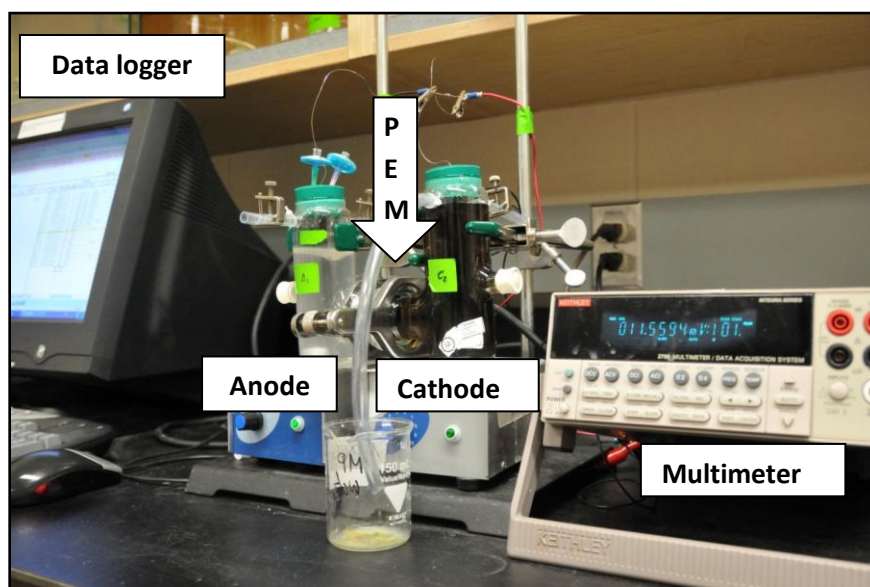


Figure 4.5 Dual chamber microbial fuel cell - data logger, anode, proton exchange membrane (PEM), cathode, and multimeter (left to right).

The medium containing the ammonia (anodic chamber) was purged with nitrogen gas for 15 minutes to remove the oxygen and create anaerobic conditions. Inoculum consists of cell pellets, separated from 100 mL culture that was resuspended in 1 mL RO water. Following the inoculation and progression of the ammonia oxidation process, various resistors (100, 500, 1000, 2000, 2400, and 6000 ohms) were placed between the anode and the cathode to determine the current and power density. Once the circuit was complete, the voltage was allowed to stabilize again. This steady state voltage value was used to calculate the current according to Ohm's law (equations 4.1 and 4.2).

$$V = IR \quad 4.1$$

$$P = VI \quad 4.2$$

After the steady state voltage was established the resistor was removed and the reactor was once again allowed to reach steady open circuit voltage. This procedure was repeated for all of the resistors. After each sample was taken, equal volume of medium with no ammonia was added to make up for the lost volume. The level of the anolyte was continuously recorded in order to determine the surface area of the anode exposed to anolyte, which was used to determine power density (equation 4.3).

$$\frac{P}{SA} = \frac{VI}{SA} \left(\frac{W}{m^2} \right) \quad 4.3$$

P (power): Watts (W); I (current): Amps (A); V (electric potential): Volts (V); SA (anode surface area): m²

4.3.3.1 *Batch operation of MFC: Effect of ammonia concentration*

Medium containing 7, 18 and 36 mM NH₄⁺-N were tested to evaluate the effect of ammonia concentration on oxidation of ammonia (nitrification) and generation of electricity. The anode was sampled every two days to track the change in ammonia, nitrite and nitrate concentrations as a function of time. Current was measured as described in the previous section. Each trial continued until all the ammonia and produced nitrite were removed. Some of the experiments were run in sequence. This means that after the initial substrate was fully oxidized, second and third batches of substrate (concentrated ammonia solution) was added consecutively to the same anode.

A number of batch runs was carried out using resazurin as an electron mediator. An effective mediator should: 1) be soluble in the anolyte, 2) be able to cross the cell membrane, 3) be able to grab electrons from the electron transport chain, 4) have a fast electrode reaction rate, 5) be non-biodegradable and non-toxic to the bacteria, 6) and have low cost (Du et al., 2007). Sund et al. (2007)

compared resazurin as an MFC mediator to safranin O, methylene blue, humic acid, and 9,10-anthraquinone-2,6-disulfonic acid disodium salt (AQDS) and found that while resazurin increased the current production, it did not seem to affect the metabolic products. Findings by Sund et al. (2007) have suggested that resazurin is able to pass through the phospholipid layer more easily and readily than other mediators and they have attributed this characteristic to the charge-less and planar structure of the dye allowing it to easily pass through the cell membrane.

Resazurin dye has been used for over 50 years to determine bacterial growth and toxicity through its changes in color from dark blue resazurin to pink resorufin and eventually colorless hydroresorufin as a result of oxidation reaction occurring in the environment (O'Brien et al., 2000). The chemical structure of resazurin and resorufin can be seen in Figure 4.6. Resazurin has no toxic effects on various bacterial cultures even over a long period of use and it has been shown to be fast, sensitive, and stable measurement of cell viability through redox reaction (O'Brien et al., 2000). These characteristics just mentioned, made resazurin a good mediator to use in this MFC setup.

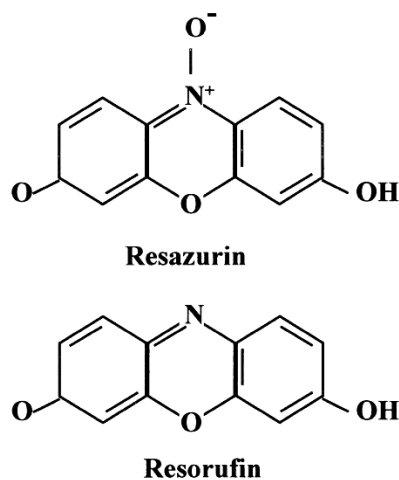


Figure 4.6 Mediator dye resazurin is deep blue in its oxidized state but turns into pink resorufin form due to reduction (O'Brien et al., 2000).

All the conditions for the microbial fuel cell operation have been kept the same while concentrated resazurin was added to the anodic medium to the final concentration of 1 mM. The concentration was chosen based on Wilkinson et al. (2001) study where 1 mM electron mediators, such

as methylene blue and neutral red, were found to improve the performance of the microbial fuel cell that was utilizing 50 mM glucose. Additionally, it was found that a 50/50 mix of methylene blue and neutral red with the total mediator concentration of 1 mM yielded the best power density and energy efficiency when compared to 1 mM of either mediator on their own (Wilkinson et al., 2006). It was speculated that the location of the bacteria with which the dye interacts is one of the determining factors for the effectiveness of the dye and the dyes may interact differently depending on the type of cell (Wilkinson et al., 2006). While it is not clear what part of the bacterial cell the resazurin dye interacts with, diaphorase enzymes (also known as dihydrolipoamine dehydrogenase, NAD(P)H:quinone oxidoreductase, and flavin reductase) are thought to be the most likely reducers of resazurin and these enzymes are found in a variety of different species in either the mitochondria or the cytoplasm (O'Brien et al., 2000).

4.3.3.2 Batch operation of MFC: Effect of nitrite concentration

Second substrate tested in the anode was nitrite at concentrations of 8, 18, 37, and 62 mM NO_2^- -N. The anode was sampled every two days to measure the nitrite and nitrate concentrations over time. Current was measured as described in the previous section. The batches were stopped when all the nitrite was removed. Many of the nitrite batch trials were run in sequence. After the first batch was complete, the second batch was started in the same anode, once the second batch of nitrite was removed, the third batch of nitrite was added and oxidized. In some of the batches resazurin was used as an electron mediator and just as in the ammonia batches, 1 mM resazurin was injected into these batches.

4.4 Analyses

The batch samples were taken directly from the reactor, while the continuous samples were taken from the sampling ports on the reactor using syringes. Samples were taken from the reactor once or twice a day depending on the necessity. Care was taken to take representative samples, this meant mixing the reactor well and redistributing cells collected in dead zones and surfaces before taking a sample. In the continuous system, the samples from the incoming feed was taken and analyzed daily, to ensure that there is no feed contamination and the substrate concentration in the feed stream remained at the desired level.

Sample (0.7 mL) was centrifuged at 9180xg (Microfuge 18 Centrifuge, Beckman Coulter, USA) for 5-10 minutes in order to separate the biomass. The supernatant was used for the ammonia, nitrite, and nitrate measurements while the cell pellets were used for the protein measurement. Diluted samples were stored in the -80°C deep freezer for possible future analysis.

4.4.1 Measuring ammonia

The Nessler method, a colorimetric assay, was used to measure the ammonia concentration (Nessler Reagent kit, Hach Co., USA). For each ammonia measurement, 5.0 mL of sample was needed. The range of ammonia concentration which could be measured by this method is between 0.02 to 2.5 mg $\text{NH}_4^+\text{-N}$ /L. Therefore all samples were diluted at least 10 times, which reduced the required sample volume from the reactor to 0.5 mL for each test. Only the supernatant portion of the centrifuged sample was used for this test. The sample taken from the bioreactor (0.5 mL) was serially diluted with reverse osmosis (RO) water until the ammonia content of the diluted sample was within the range for the Nessler method.

Two drops of the mineral stabilizer from the kit was added to all the samples including the blank, followed by two drops of polyvinyl alcohol dispersing agent which resulted in color development in each sample. After mixing the sample, 0.5 mL of Nessler Reagent was added. In the presence of ammonium the sample will turn yellow, the blank sample should stay clear. Samples were left for one minute, and then the optical density of the samples (yellow color) was determined at 425 nm using a spectrophotometer (UVmini-1240 spectrophotometer, Shimadzu, Japan). Reverse Osmosis (RO) water was used as the blank. The blank sample was used to zero the spectrophotometer before reading the actual samples. The colors of the samples change over time, so it was important to measure the optical density on a consistent basis (one minute after addition of reagent). The optical density was then converted to ammonia concentration (mg/L) using a calibration curve developed with standard ammonia solution (see Appendix A).

4.4.2 Measuring nitrite and nitrate

The concentrations of nitrate and nitrite were measured simultaneously using an ion chromatograph (ICS-2500, Dionex Co., USA). Only 0.1 mL of sample supernatant was needed for each nitrite/ nitrate measurement. The sample was diluted by 0.9 mL Millipore water, this diluted sample was further diluted to the desired concentration range based on the IC calibration (5 – 40 ppm of nitrite or nitrate).

4.4.3 Measuring protein

The nitrifying biomass concentration was extremely hard to measure using conventional methods such as optical density and dry weight because of their slow growth and the tendency of the cells to form flocks. As a result, a protein measurement was used as a way to indirectly determine the biomass concentration in the reactor. According to Shuler and Kargi (2001), about 50% of a bacterial cell

mass is protein. This relationship was verified by Campos et al. (1999) when they showed that the ratio between the volatile suspended solids and the total protein concentration is 2 g VSS to 1 g protein for nitrifying biomass. Therefore, a direct correlation between the total protein concentration and the biomass concentration can be made. However, due to the limited volume of the reaction liquid in the batch system and the relatively large volume of samples required for the protein measurement, these measurements were only carried out in the continuous reactors.

The Bradford method was used to determine the protein concentration (Coomassie Plus Protein Assay Reagent, ThermoFisher Scientific, USA). The cell pellets from the centrifuged sample were completely separated from the supernatant and resuspended in 1 mL RO water. This suspension was then sonicated for 3 minutes at 20 watts to promote cell lysis. The sample was kept in the cold water bath while sonicating, to absorb the heat from the process and prevent protein degradation. The lysed sample was then used for the Bradford assay. The kit used in this work was suitable for two concentration ranges of 0 – 25 mg/L and 25 – 750 mg/L. For the higher range, only 0.05 mL of sample was needed, while with the lower 1.0 mL sample was used.

Brownish colored Coomassie reagent was added to each sample and a blank sample (RO water), then these samples were left for 10 minutes for the color development. The absorbance of the samples was then measured at 595 nm using a spectrophotometer (UVmini-1240 spectrophotometer, Shimadzu, Japan). The spectrophotometer was zeroed using the sample with the RO water. The absorbance can be used to determine the protein concentration using standard calibration curves prepared with standard bovine serum albumin solutions (see Appendix A).

5 RESULTS AND ANALYSIS

5.1 Ammonia removal in batch systems

The first part of the batch study looked at the effects of initial ammonia concentration on the removal of ammonia, generation of nitrite, and conversion of nitrite to nitrate in the free-cell and biofilm reactors. The bacterial concentration in the free cell batch trials was not measured because of the slow growth rate of the culture and the tendency to flocculate, making OD unreliable.

5.1.1 Batch reactor with free cells

Figure 5.1 shows the results of ammonia oxidation at different initial concentration. The initial ammonia concentrations tested were 5 mM (Figure 5.1 A), 15 mM (Figure 5.1 B), 36 mM (Figure 5.1 C), and finally 73 mM (Figure 5.1 D). Data represent the average value obtained in duplicate experiments and error bars are the associated standard errors. The first few trials from the acclimation period are not included in Figure 5.1. In all cases, the ammonia concentration decreased as it got oxidized to nitrite, and once all the ammonia was oxidized, the nitrite was further oxidized to nitrate. There was 100 percent removal of both ammonia and the generated nitrite in all the trials for ammonia at concentrations up to 73 mM (1000 PPM). The time it took for ammonia to oxidize to nitrite increased from about 70 hours to 300 hours, when initial concentration was increased from 5 to 73 mM, and the time for nitrite to oxidize to nitrate increased from about 130 to 230 hours. After complete ammonia oxidation, the nitrite concentration was less than what was expected from the stoichiometry; however the final nitrate concentration matched the value expected. For the range of tested concentrations no obvious inhibition was observed. There was a marked decrease in ammonia oxidation rate due to reactor pH drop from the high concentration ammonia oxidation and the resulting produced protons.

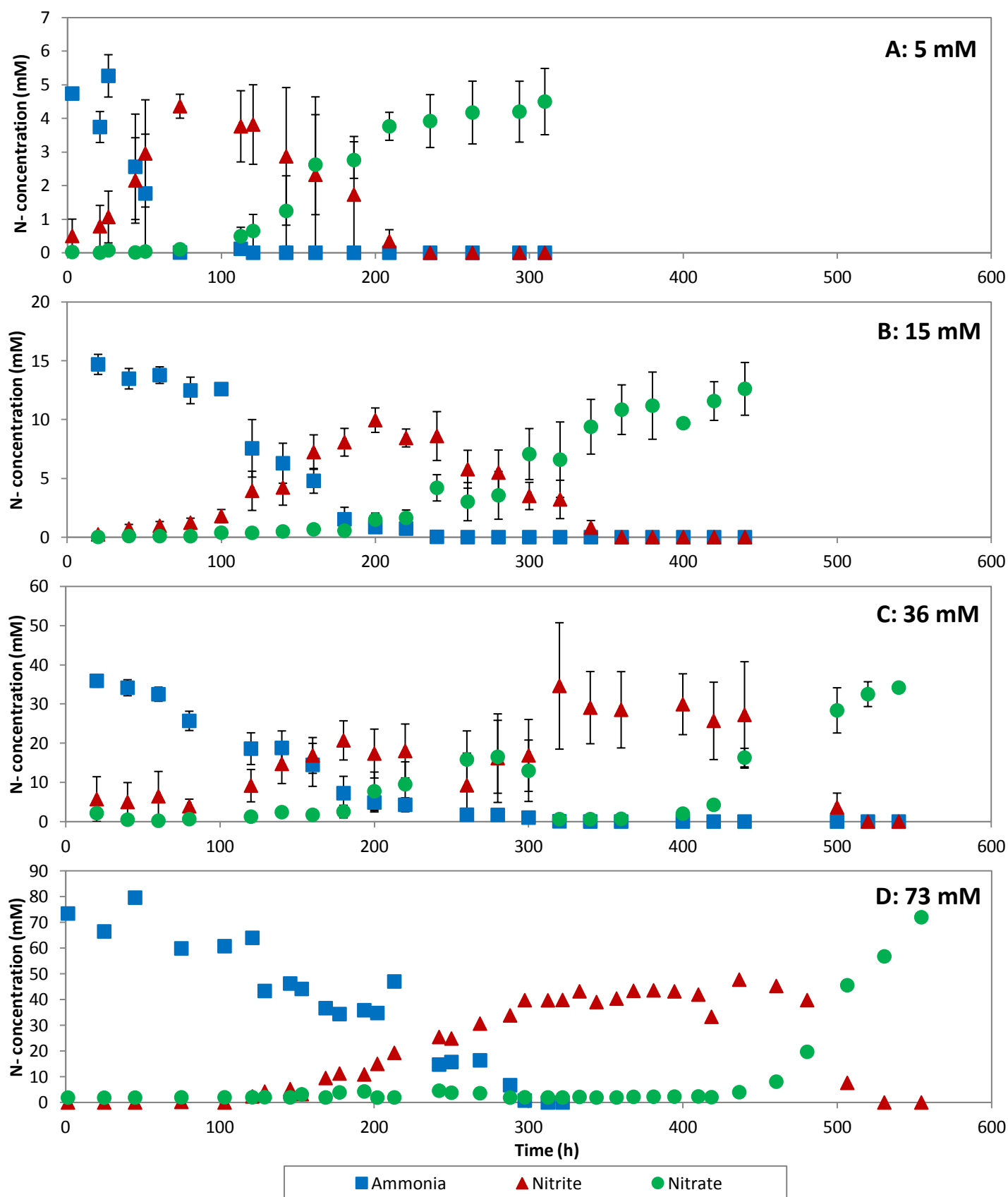


Figure 5.1 Concentration of various ions during oxidation of 5 (A), 15 (B), 36 (C), and 73 (D) mM initial ammonia concentration in free cell batch reactors. Data represent the average value of concentrations from duplicate experiments and error bars are the associated standard errors.

5.1.2 Batch reactor with biofilm

The ammonia, nitrite, and nitrate profiles for the biofilm batch reactors closely follow those of the free-cell reactor. The sequential oxidation of ammonia followed by nitrite oxidation was also seen in the biofilm reactors. Figure 5.2 summarizes the concentration profiles for oxidation of ammonia at concentrations of 3, 15, 35, and 100 mM in the biofilm reactors. Some reactors contained porous ceramics and others contained filter foam as support. The data from reactors containing both ceramic and filter foam media are included in this analysis.

The first trial in the biofilm reactors were the 3 mM batches which took nearly 400 hours to completely oxidize to nitrate, in contrast to the 200 hours in the free cell reactor. At this early stage, biofilm did not have time to form on the matrix so the ceramics and the foam were hindering the oxidation reaction by the small amount of nitrifying cells in the reactor by effectively shielding access to the substrate. Each consecutive batch (Figure 5.2 B, C, D) took shorter time to oxidize than the one before because the biofilm is building up over time so the higher biomass concentration is helping to oxidize the substrate faster and offsetting whatever mass transfer limitations caused by the ceramics or foam. At 100 mM ammonia concentration in the biofilm reactor (Figure 5.2 D), the nitrate concentration only reached 60 mM.

The time it took for ammonia oxidation stayed nearly the same at 200 hours for both 3 mM (Figure 5.2 A) and 15 mM (Figure 5.2 B) concentrations, but decreased to 170 hours for 36 mM (Figure 5.2 C) and 100 mM (Figure 5.2 D). The time for nitrite oxidation was 200 hours at 3 mM, 120 hours at 15 mM, and 70 hours at 36 mM and 100 mM. The buildup of nitrite in the reactor in proportion to the starting ammonia concentration is decreasing during the same period. After enough time was given for the biofilm to develop on the matrix, the biofilm reactors were able to oxidize at a faster rate than the free cell reactors, as seen by the time each batch took to completely oxidize the ammonia and nitrite.

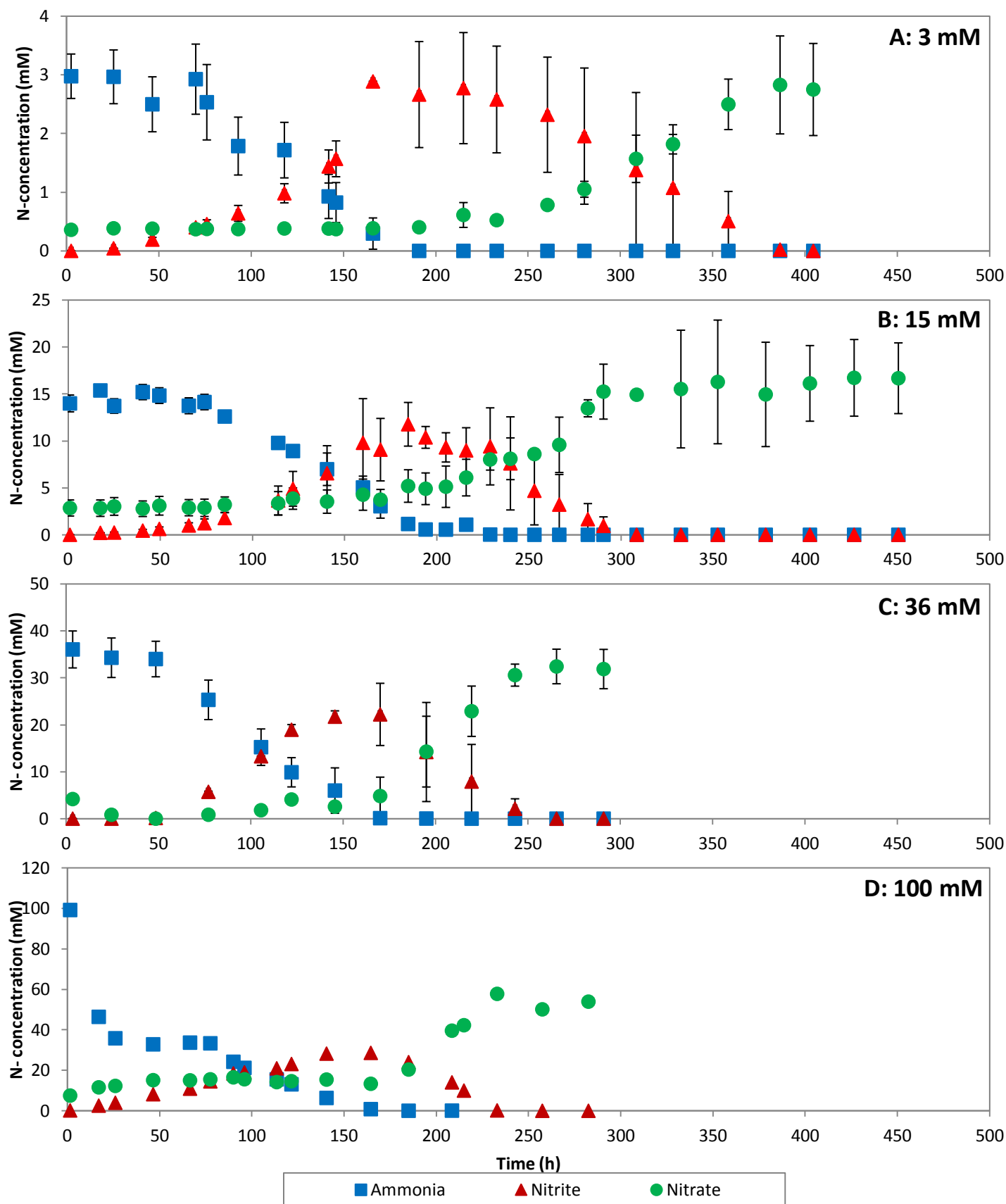


Figure 5.2 Concentration of various ions during oxidation of 3 (A), 15 (B), 36 (C), 100 (D) mM ammonia in biofilm batch reactors. Data represent the average value of concentrations from duplicate experiments and error bars are the associated standard errors.

5.2 Nitrite removal in batch systems

In the second part of the batch trials, nitrite oxidation was investigated. This was in order to study and compare oxidation of biologically produced nitrite (result of ammonia oxidation) with nitrite as the sole substrate. Total of 23 free-cell batch trials were carried out using 3, 6, 15, 30, and 60 mM nitrite as the original substrate. Due to time limitation oxidation of nitrite as the sole substrate was not investigated in the biofilm system.

5.2.1 Nitrite oxidation batch reactors with free cell

Figure 5.3 shows the profile of nitrite and nitrate concentrations obtained in the batch reactors with free cells. Oxidation of 6 and 15 mM nitrite (Figure 5.3 A and B) took about 75 hours to complete, while oxidation of 30 mM (Figure 5.3 C) and 60 mM (Figure 5.3 D) nitrite took 120 and 140 hours, respectively. Oxidation of nitrite as the original substrate was faster than that of biologically produced nitrite observed in the free cell reactor. For instance oxidation of 15 mM (250 ppm) nitrite as the original substrate (Figure 5.3 B) took 75 hours to complete, while with 10 mM biologically produced nitrite, 150 hours were required (Figure 5.1 B). The difference in observed oxidation rates may be attributed to the competitive growth advantage ammonia oxidizers have over nitrite oxidizers. In a free cell reactor, with nitrite as sole substrate, the nitrite oxidizer population grew larger than what was possible in the reactor fed with ammonia.

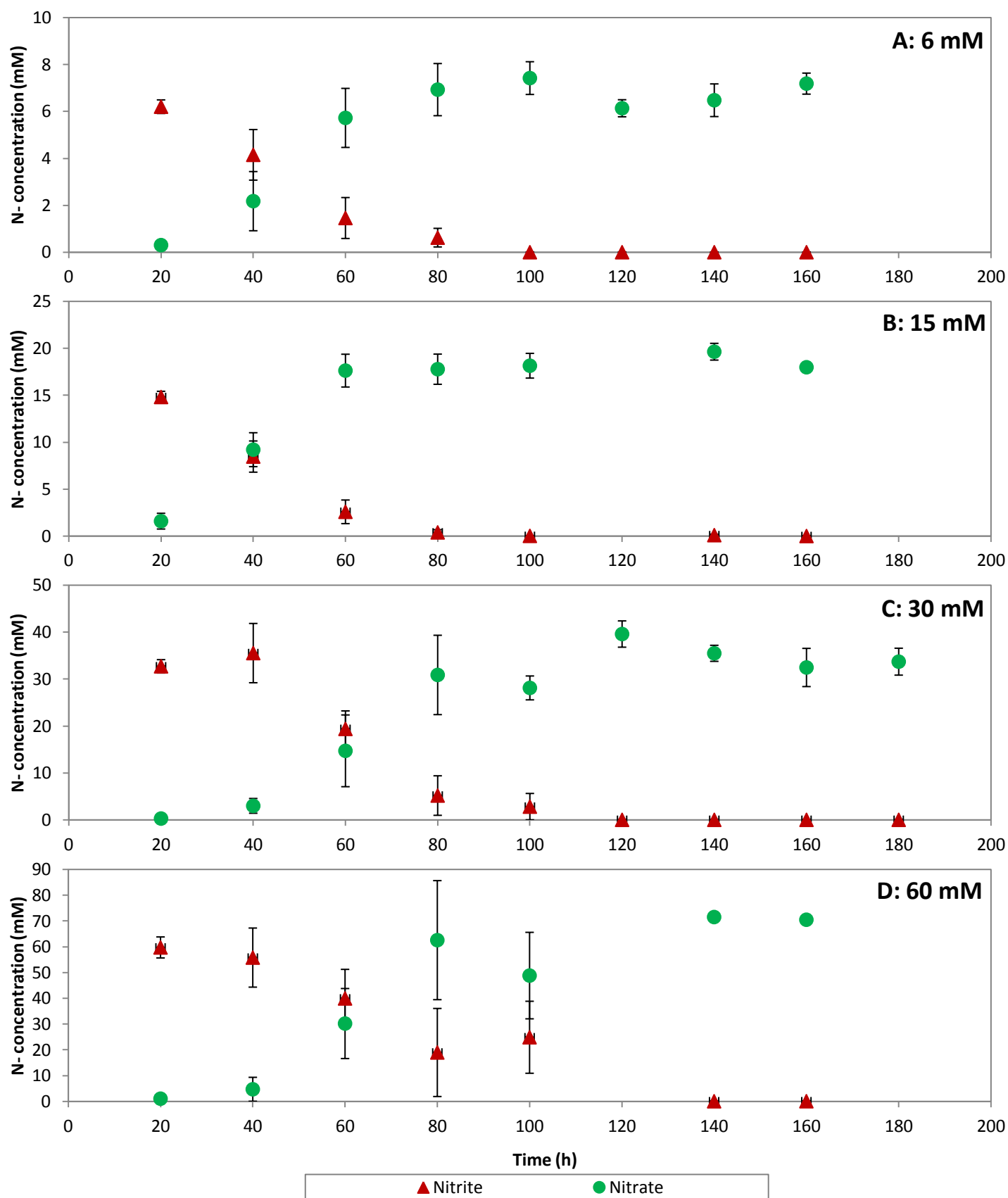


Figure 5.3 Concentration of various ions during oxidation of 6 (A), 15 (B), 30 (C), and 60 (D) mM nitrite in batch reactors with free cells. Data represent the average value of concentrations from duplicate experiments and error bars are the associated standard errors.

5.3 Rate of oxidation in batch system

The rate of ammonia oxidation was determined by calculating the slope of the linear part of ammonia concentration curve and as the nitrite starts to build up in the reactor (lag phase was excluded). The ammonia substrate concentration is the average of the ammonia concentration measurements at the start of the experiment before the oxidation takes place. The rate of nitrite oxidation was calculated separately in free cell and biofilm trials and compared against the rate of oxidation when nitrite was the sole substrate. Nitrite oxidation rate was determined by taking the slope of the linear part of nitrite concentration curve and as nitrate production started (second lag phase was excluded). The nitrite concentration of trials using sodium nitrite is determined by the average concentration measurement of nitrite before oxidation starts while the biological nitrite produced by ammonia oxidation is determined by taking the highest concentration of nitrite produced before the downward slope of oxidation.

5.3.1 Ammonia oxidation rate

Figure 5.4 shows the oxidation rate of ammonia as a function of its initial concentration for biofilm and free cell cultures. The oxidation rate data as well as the initial substrate concentration data of ammonia in biofilm (Figure 5.4 A) and in free cell cultures (Figure 5.4 B) have been averaged and the standard error bars for the concentration and the oxidation rate is given. The average range of ammonia concentrations tested for the biofilm is between 3 to 66 mM while the concentrations tested for free cell culture was between 3 to 67 mM.

The biofilm system was adapted over time to sustain increasingly larger number of nitrifying bacteria, while the free cell cultures were limited by the amount of bacteria in the 10 mL inoculum volume from the previous batch to the new one. In both cultures, ammonia oxidation rate is directly

correlated with the initial ammonia concentration over the range of tested concentrations. The regression lines on Figure 5.4 demonstrate the linear increase in oxidation rate as ammonia concentration was increased.

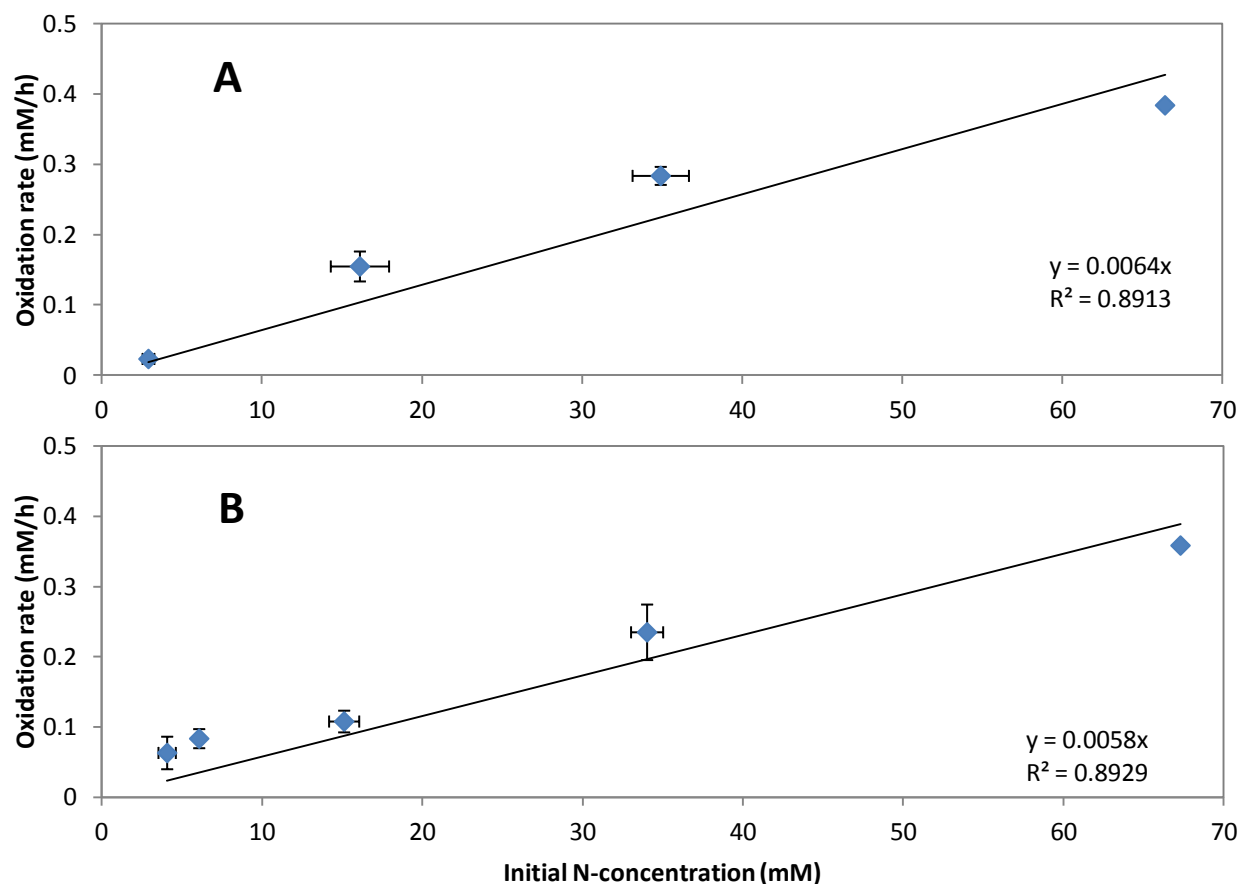


Figure 5.4 Ammonia oxidation rate for A) biofilm and B) free cell reactors. Vertical error bar represents the standard error in the calculated oxidation rate and the horizontal bar represents the standard error in the measured initial concentration for duplicate biofilm and free cell trials.

Furthermore, this increase is slightly larger for biofilm reactors (Figure 5.4 A) in comparison to free cell reactors (Figure 5.4 B), as the slope of the fitted lines are 0.0064 h^{-1} and 0.0058 h^{-1} , respectively. The highest oxidation rate reached by the biofilm reactor is about 0.38 mM/h at 66 mM substrate concentration while the free cell culture reached 0.36 mM/h at 67 mM . The regression line for both data has coefficient of determination of 0.89 , the region of data under 40 mM is extremely linear while at higher concentrations there may be a start of a leveling off effect.

5.3.2 Nitrite oxidation rate

Figure 5.5 contains the oxidation rate data of biological nitrite from ammonia oxidation and sodium nitrite as a function of starting substrate concentration. The rate and the substrate concentration values are averages of multiple trials, therefore the standard error bars are given in each direction. There were three conditions under which nitrite oxidation was studied: 1) biofilm reactor with biological nitrite as substrate between 3 to 29 mM (Figure 5.5 A), 2) free-cell reactor with biological nitrite as substrate between 3 to 45 mM (Figure 5.5 B), and 3) free cell reactor with sodium nitrite as substrate between 3 to 65 mM (Figure 5.5 C). In all three cases the nitrite oxidation rate increased linearly with increases in initial nitrite concentration and the regression lines represent this dependency. In case of biological nitrite in biofilm and free cell cultures, the R^2 values were 0.9 and 0.92 respectively, while the sodium nitrite R^2 was 0.98. The oxidation rate of sodium nitrite as substrate reached 1.2 mM/h where the rate constant was 0.019h^{-1} , followed by free cell oxidation rate of 0.52 mM/h with the slowest rate constant of 0.013h^{-1} , and finally biofilm reactor rate of 0.42 mM/h at the rate constant of 0.017h^{-1} .

Similar to what was observed with ammonia, the rate of biological nitrite oxidation (Figure 5.5 A and B) had very linear relationship with substrate concentration up to about 25 mM. At concentrations greater than 25 mM, the rate increase seems to wane and become less linear. On the other hand, the sodium nitrite oxidized linearly all the way up to 65 mM with no sign of leveling off. This possible saturation effect of the biological nitrite oxidation resulting in maximum oxidation rate lower than what seems to be possible with sodium nitrite might be an indication of an inhibition by ammonia oxidizers in the same reactor. Having more data points in these ranges would make the saturation effect, if truly present, more evident.

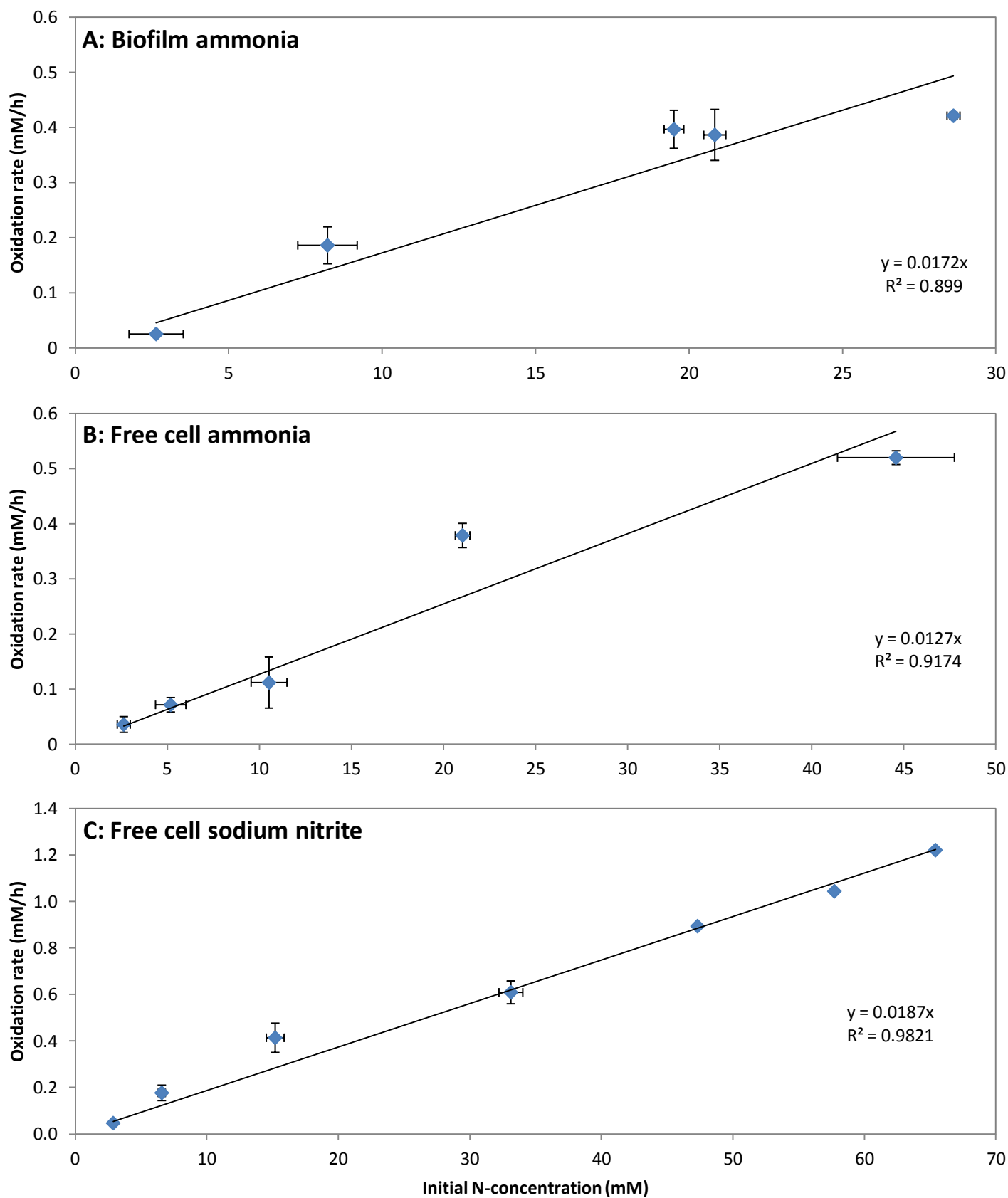


Figure 5.5 Nitrite oxidation as a function of initial nitrite concentration – A) biofilm reactor, starting substrate ammonia, B) free cell reactor, starting substrate ammonia, and C) free cell reactor, starting substrate nitrite. Vertical error bar represents the standard error in the oxidation rate and the horizontal bar is the standard error in initial concentration for duplicate trials.

5.3.3 Comparison of ammonia and nitrite oxidation rate

Figure 5.6 compares the ammonia and nitrite oxidation rate as a function of initial concentration. By looking at this graph, there is an opportunity to compare the effect of the different culture types, biofilm vs. free cell, and different sources of nitrite, biological vs. sodium nitrite. The sodium nitrite oxidation in free cell culture was the fastest reaction and the ammonia oxidation in free cell reactor was the slowest. The free cell nitrite oxidation was twice as fast as ammonia oxidation in the same reactor, biofilm nitrite oxidation was 2.6 times faster than ammonia oxidation in the same reactor, and the sodium nitrite oxidation in free cell was almost three times faster than ammonia oxidation in free cell culture. Sodium nitrite oxidation rate was comparable with biological nitrite oxidation in biofilm culture while the biological nitrite oxidations in free cell cultures were about 50% slower than either of these reactions. The ammonia oxidation in biofilm culture also seems comparable to ammonia oxidation in a free cell culture.

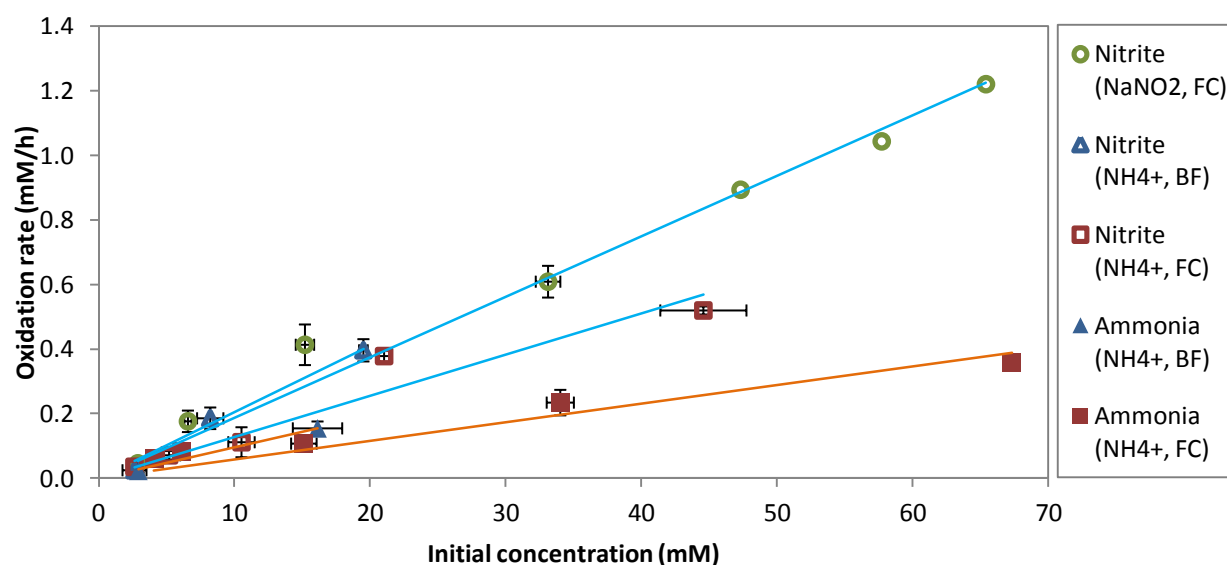


Figure 5.6 Average ammonia and nitrite oxidation data as a function of initial nitrogen concentration. In the parenthesis, the first variable denotes the type of starting substrate in the batch (sodium nitrite or ammonia) and the second variable denotes the type of culture used (FC- free cell, BF- biofilm). The regression lines in blue are for oxidation of nitrite and in orange are for oxidation of ammonia.

In a reactor where both ammonia and subsequently nitrite were being oxidized, the oxidation rate of nitrite seems to be slower than what is possible when nitrite is oxidized alone. This may be due

to inhibition from ammonia oxidizers. On the other hand, the biological nitrite oxidation in the biofilm culture does not seem to suffer from inhibition since the rate is comparable with the sodium nitrite oxidation rate. Though the biofilm reactor may not experience inhibition due to its larger microbial population, it may experience mass transfer limitations due to its structure.

5.4 Ammonia removal in continuous systems

The second phase of the study looked at the continuous operation of free-cell and biofilm reactors using ammonia as substrate. The main factors being investigated were the impact of ammonia loading rate on the contaminant removal rate and the composition of end products. Loading rate of the reactors was controlled by varying the feed flowrate at constant ammonia concentration. This approach was applied at three different feed ammonia concentrations. In both the CSTR and biofilm reactors, the cells took some time to adjust to the new condition after each change in flowrate or feed concentration. The feed ammonia concentration was measured daily to ensure the medium was not contaminated and the effluent ammonia, nitrite, and nitrate were measured multiple times a day once it was determined that the reactor has reached steady state. Steady state conditions were characterized by limited change (less than 10%) in the effluent ammonia, nitrite, and nitrate concentrations. Figure 5.7 shows an example of the transient period in a biofilm reactor followed by the steady state condition when the reactor was fed 15 mM ammonia at a flow rate of 10 mL/h. Only the data collected during the steady state condition were used to evaluate the performance of the reactor. After the steady state data were collected, the feed flowrate or concentration was increased to the next level.

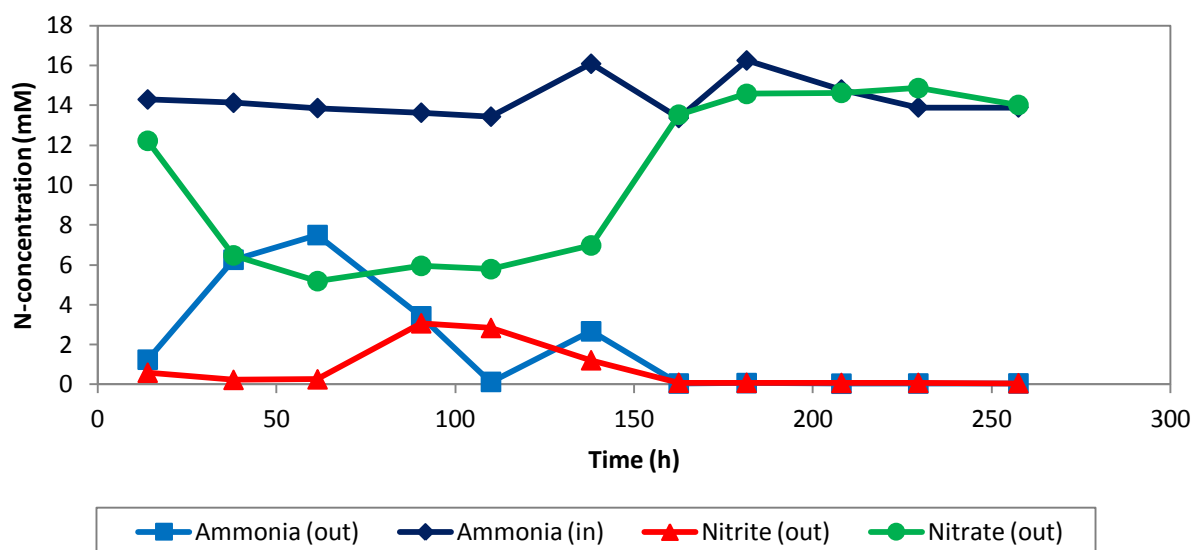


Figure 5.7 Transient data in biofilm reactor operated with 15 mM ammonia at feed rate of 10 mL/h.

5.4.1 Ammonia removal rate as a function of loading rate

With a constant feed concentration (17.6, 31.7, and 62.3 mM) and where the flowrate was used to change the loading rate, ammonia removal rate increased with increase in loading rate in both CSTR and biofilm until it leveled off in the biofilm reactor (Figure 5.8 B) and started to decrease in the CSTR (Figure 5.8 A). The profile of the ammonia removal rate vs. its loading rate displayed the same pattern with all three feed concentrations tested. The CSTR experienced increase in removal rate as loading rate was increased for loading rates up to 4 mM/h. Further increase in loading rate led to decrease in removal rate.

The biofilm reactor displayed the same trend of increasing removal rates with loading rate up to 8 mM/h before the removal rate reached a plateau as the loading rate increased. Feed concentration had little effect on the removal rate in biofilm reactor (Figure 5.8 B). On the other hand, the ammonia removal rate seems to slightly decrease as the feed concentration increased in the CSTR (Figure 5.8 A). The highest removal rate in the CSTR was 3.4 mM/hr at 5.4 mM/h loading rate while the fastest removal rate reached in the biofilm reactor was 8.8 mM/h at 20.4 mM/h loading rate. The biofilm reactor was

able to achieve more than twice the removal rate at nearly four times the loading rate of the CSTR. The CSTR might be more prone to experience inhibition effects as a result of high ammonia or byproduct concentration. The biofilm reactors might be less affected by the high concentration because of the larger microbial population, faster ammonia removal rate, and partial protection of cells due to immobilization in the matrix.

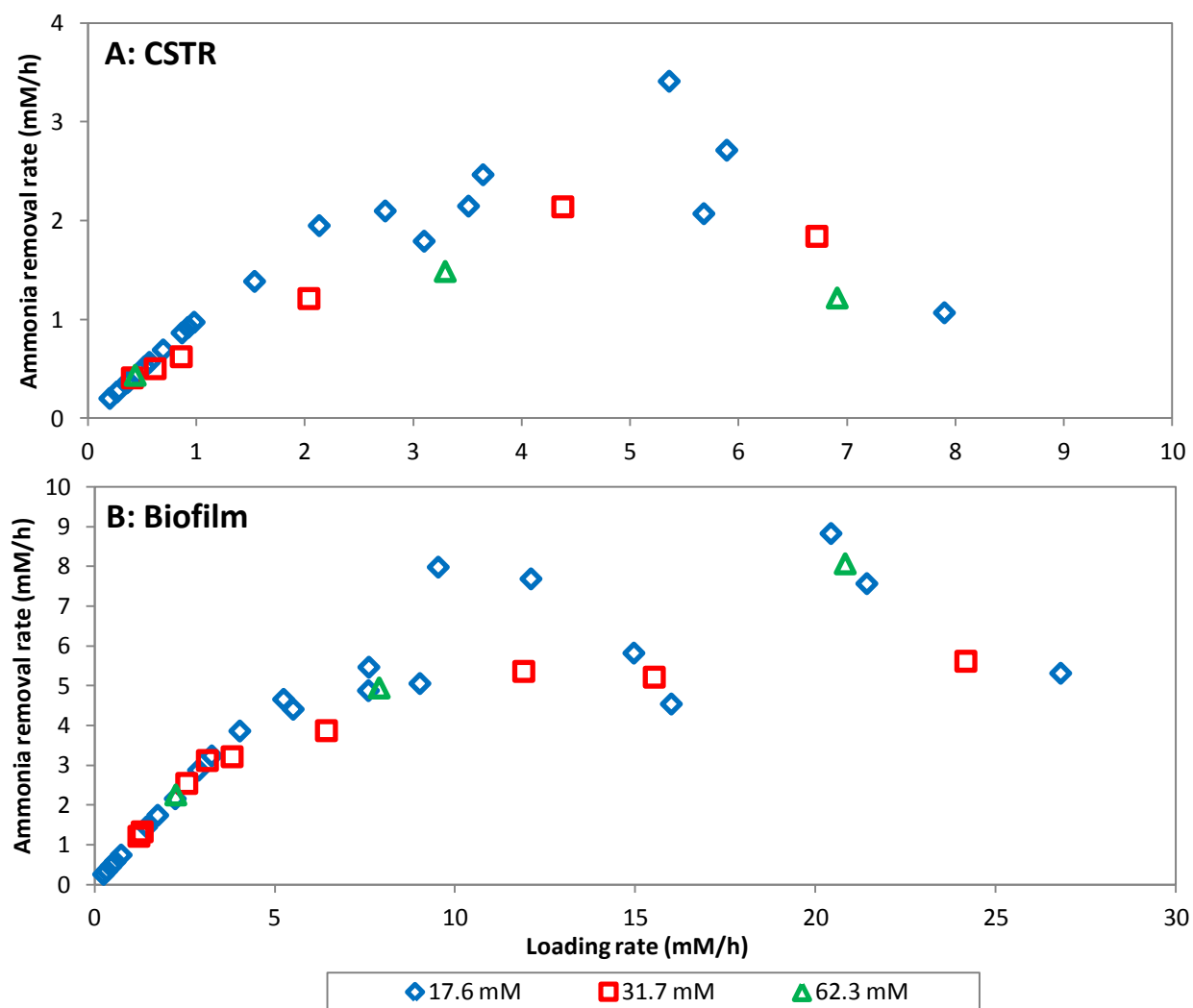


Figure 5.8 Ammonia removal rate as a function of its loading rate for A) CSTR and B) biofilm reactors.

5.4.2 Effluent composition in CSTR

As the loading rate increased, the effluent composition in the reactor changed to contain various combinations of ammonia, nitrite, and nitrate. Figure 5.9 shows the portion of the effluent nitrogen in the CSTR reactor that is ammonia (Figure 5.9 A), nitrite (Figure 5.9 B), and nitrate (Figure 5.9 C) as a function of the loading rate up to the highest applied loading rate of 8 mM/h. These data highlight the importance of the loading rate (residence time) as a factor to control the composition of the effluent and end products (i.e. nitrite vs. nitrate). The control of end product composition is especially important when shortcut biological denitrification (oxidation of ammonia to nitrite and reduction of nitrite to nitrogen gas) or ANAMMOX is used for the removal of ammonia.

The effluent ammonia concentration linearly increased as the loading rate increased (Figure 5.9 A). During the same period, the effluent nitrite stayed between 30 to 60 percent (Figure 5.9 B) until the loading rate reached 6 mM, after which, the nitrite composition dropped off. While the nitrate content of the effluent quickly dropped off below 100 percent soon after the loading rate increased above 1 mM/h (Figure 5.9 C). At each feed concentration with low loading rates, there was only nitrate in the effluent as all the ammonia and nitrite were oxidized. As the loading rate increased, a steady amount of nitrite, increasing amount of ammonia and drastically declining amount of nitrate were present in the effluent. Above 5 mM/h loading rate, there were no nitrate present, nitrite content decreased from 60% to nearly zero, and the ammonia content increased to make up the rest of the effluent nitrogen.

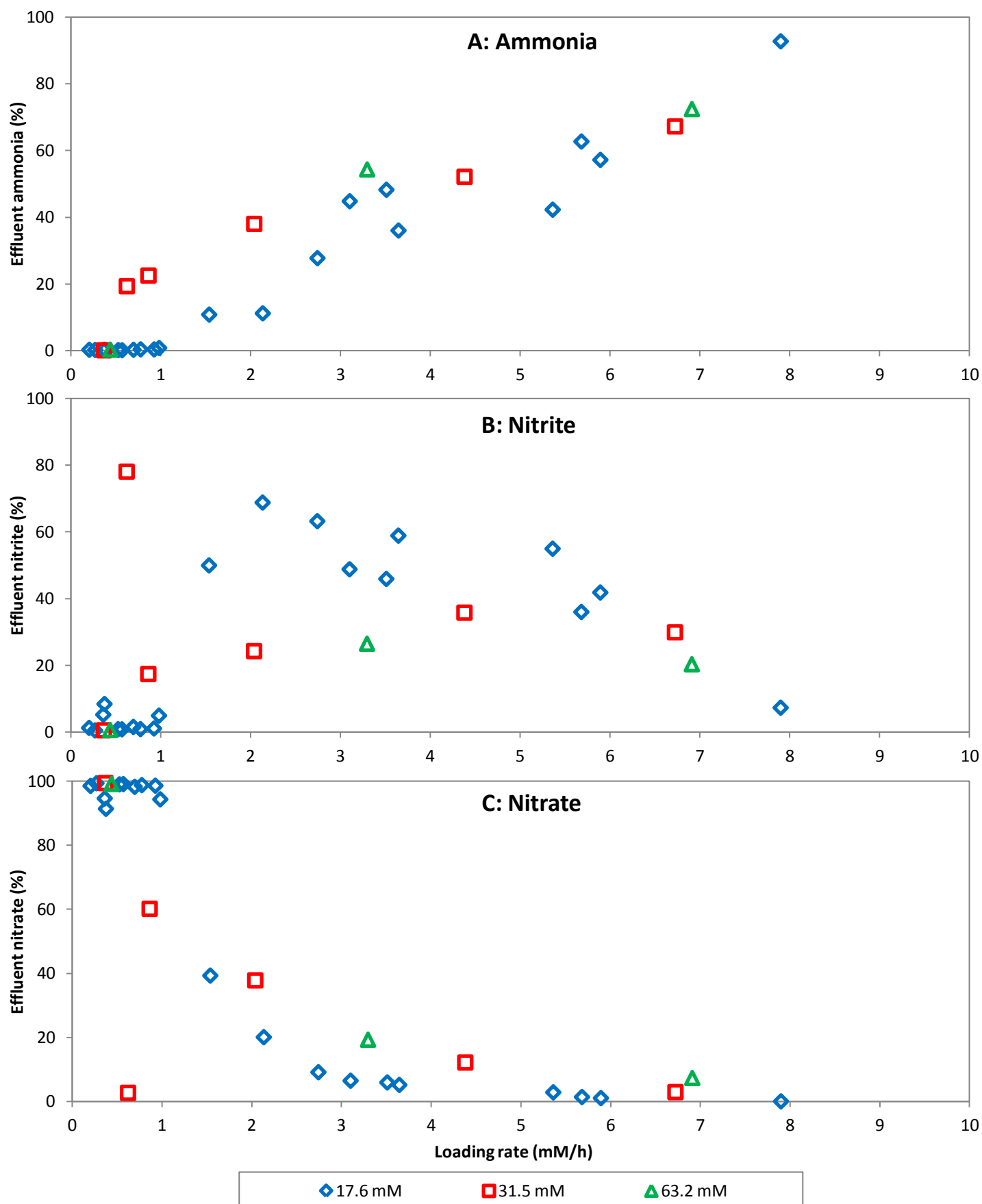


Figure 5.9 Effluent composition of CSTR, A) ammonia, B) nitrite and C) nitrate, at different feed ammonia concentration as a function of loading rate up to 8 mM/h.

There were some distinctions between the low and the two high feed concentrations. For instance, with 31.5 and 63.2 mM ammonia in the feed, residual ammonia concentration started to increase at lower loading rate than the one operated with 17.6 mM ammonia (0.5 mM/h v. 1 mM/h) possibly due to inhibition effects from the concentration. Figure 5.10 highlights the difference in the effluent compositions among the three influent ammonia concentrations, 17.6, 31.5, and 63.2 mM. With 17.6 mM ammonia (Figure 5.10 A), nitrite makes up more than 50% of the effluent content for loading rates in the range of 1 to 5 mM/h. When the loading rate is less than 1 mM/h, nitrate dominates the effluent, while for loading rates higher than 5 mM/h, ammonia is the dominant compound. With 31.5 mM/h (Figure 5.10 B), the nitrite content steadily increased from 20 to 40%, while the ammonia increased from 20 to 60% of the effluent nitrogen due to greater contaminant concentration at shorter residence time as the ammonia loading rate increased to 6 mM/h.

At 63 mM/h (Figure 5.10 C), nitrate made up vast majority of the effluent nitrogen when the loading rate was under 1 mM/h, while up to 3 mM/h ammonia quickly rose to make up 60% and nitrite slowly increased to 20%. Beyond 3 mM/h, the effluent composition stayed relatively constant around 60 – 70% ammonia, 20% nitrite, and the rest nitrate. The ammonia portion of the effluent seem to stay relatively the same across the different feed concentrations but the nitrate in the effluent does make up for the fall in nitrite portion. This is because, as the feed ammonia concentration increases, lower flowrates are needed to attain the same loading rate. So, while the slow oxidizing ammonia portion is not affected by the longer residence time, the faster oxidizing nitrite portion is further oxidized to nitrate in the higher feed concentration reactors.

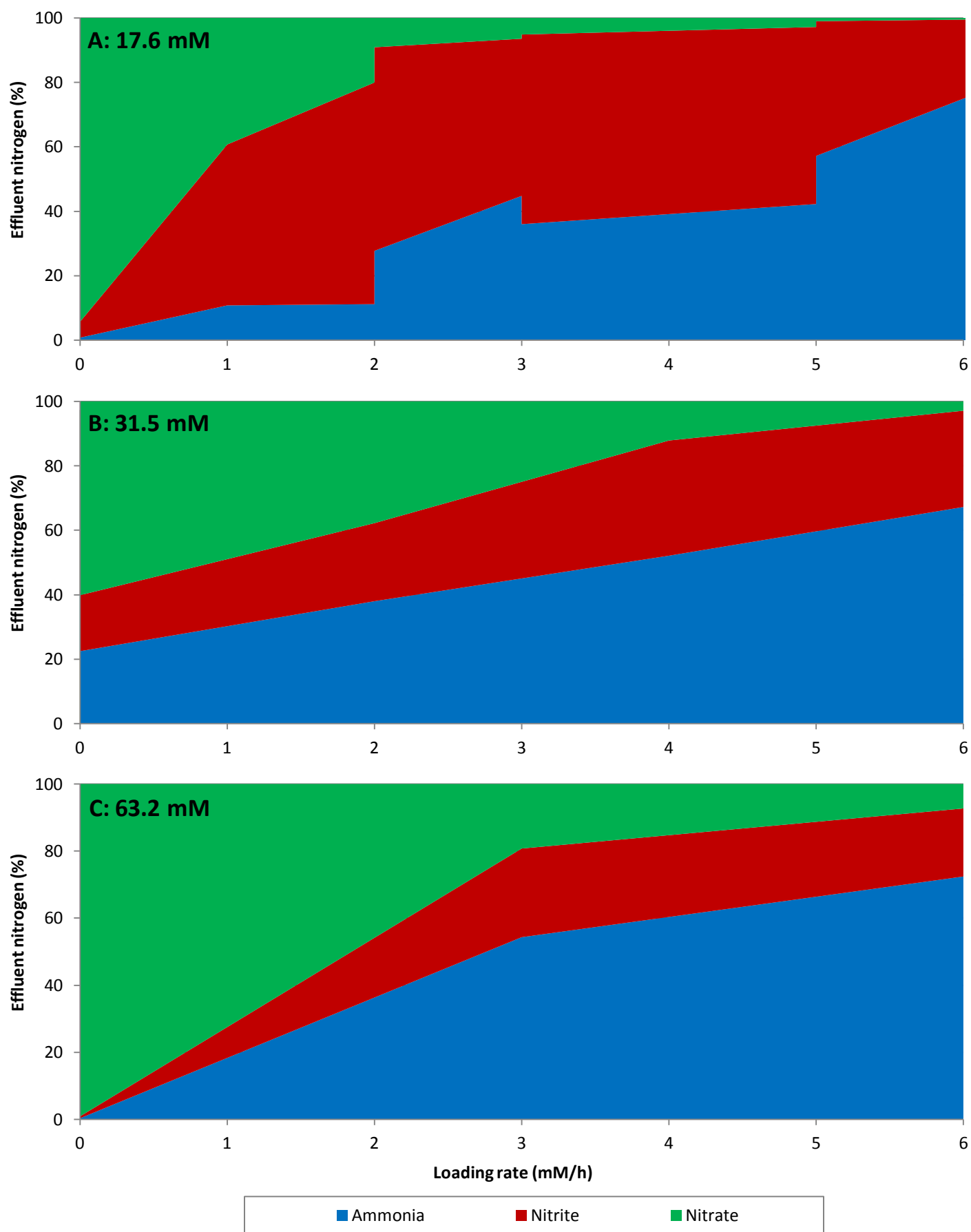


Figure 5.10 Composition of CSTR effluent nitrogen for feeds with A) 17.6 mM, B) 31.5 mM and C) 63.2 mM ammonia.

5.4.3 Effluent composition in the biofilm reactor

The biofilm reactor effluent composition was a lot more stable at different conditions therefore it was possible to run this reactor at higher loading rate of 27 mM/h (Figure 5.11). At loading rates less than 3 mM/h, the effluent was dominated by nitrate and no ammonia or nitrite was present. The ammonia content of the effluent steadily increased with increases in loading rate (Figure 5.11 A). At loading rates less than 13 mM/h, nitrite made up 40 to 80% of the effluent but fell to around 30% at higher loading rates (Figure 5.11 B). The nitrate content fell fast below 100% as the loading rate increased over 3 mM/h, with no nitrate present in the effluent past 10 mM/h (Figure 5.11 C).

There seems to be slight difference between the biofilm reactors run under different ammonia concentrations. The most noticeable difference being the reactor fed with 17.6 mM and the two higher concentrations, 31.8 and 61.5 mM, for the nitrite and nitrate composition. With 17.6 mM ammonia, the nitrate content in the effluent dropped off just as the nitrite portion drastically increased around 2 mM/h loading rate. While the 31.8 and 61.5 mM reactors were able to maintain full ammonia oxidation to nitrate up to higher loading rate (4 mM/h) than the 17.6 mM bioreactor. This indicates that even though immobilized cells are resistant to the washout effects of suspended cells, there is effect of flowrate in the biofilm reactors. This effect is most likely as a result of limited time given for full mixing and distribution of the substrate or other related mass transfer inefficiencies of the biofilm.

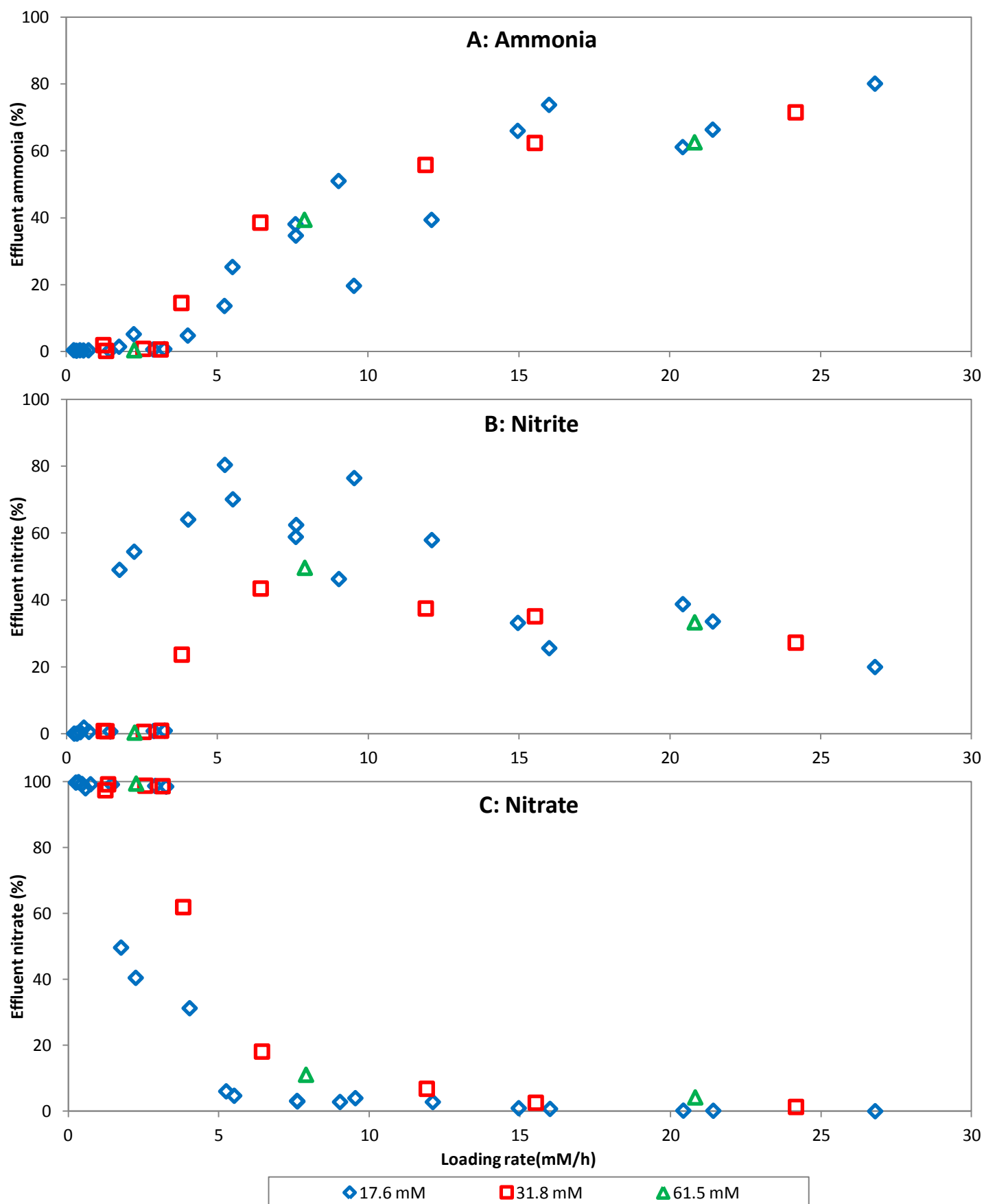


Figure 5.11 Effluent composition of biofilm, A) ammonia, B) nitrite and C) nitrate, at different feed ammonia concentration as a function of feed loading rate.

The effects of feed concentration on the effluent composition are highlighted in Figure 5.12. With 17.6 mM ammonia in the feed, the effluent was mostly composed of nitrite, 60 to 80%, and the rest of the nitrogen was ammonia when the loading rate was below 14 mM/h. With 31.8 mM ammonia, effluent was composed entirely of nitrate at loading rates below 3 mM/h. At loading rates higher than 5 mM/h, about 30 to 40% of the effluent nitrogen was nitrite and the rest was ammonia. The 61.5 mM reactor started with all nitrate and as the loading rate increased to 7 to 20 mM/h, the reactor produced a steady output of 40% nitrite and over 50% ammonia. The main pattern that emerged among the data is that at low loading rates the effluent nitrogen is all nitrate, as loading rate increases the nitrite and ammonia builds up quickly reaching a steady level and come to be composed of 40% nitrite and the rest ammonia. This type of effluent from the biofilm reactor seems to provide suitable composition for an ANAMMOX process where complete nitrogen removal takes place under anaerobic condition using 50% nitrite and ammonia in the feed. In addition to a superior performance as far as removal of ammonia is concerned, the other attractive characteristic of the biofilm reactor when compared with the free cell reactor is the fact that the effluent composition was relatively stable throughout the wide range of conditions tested.

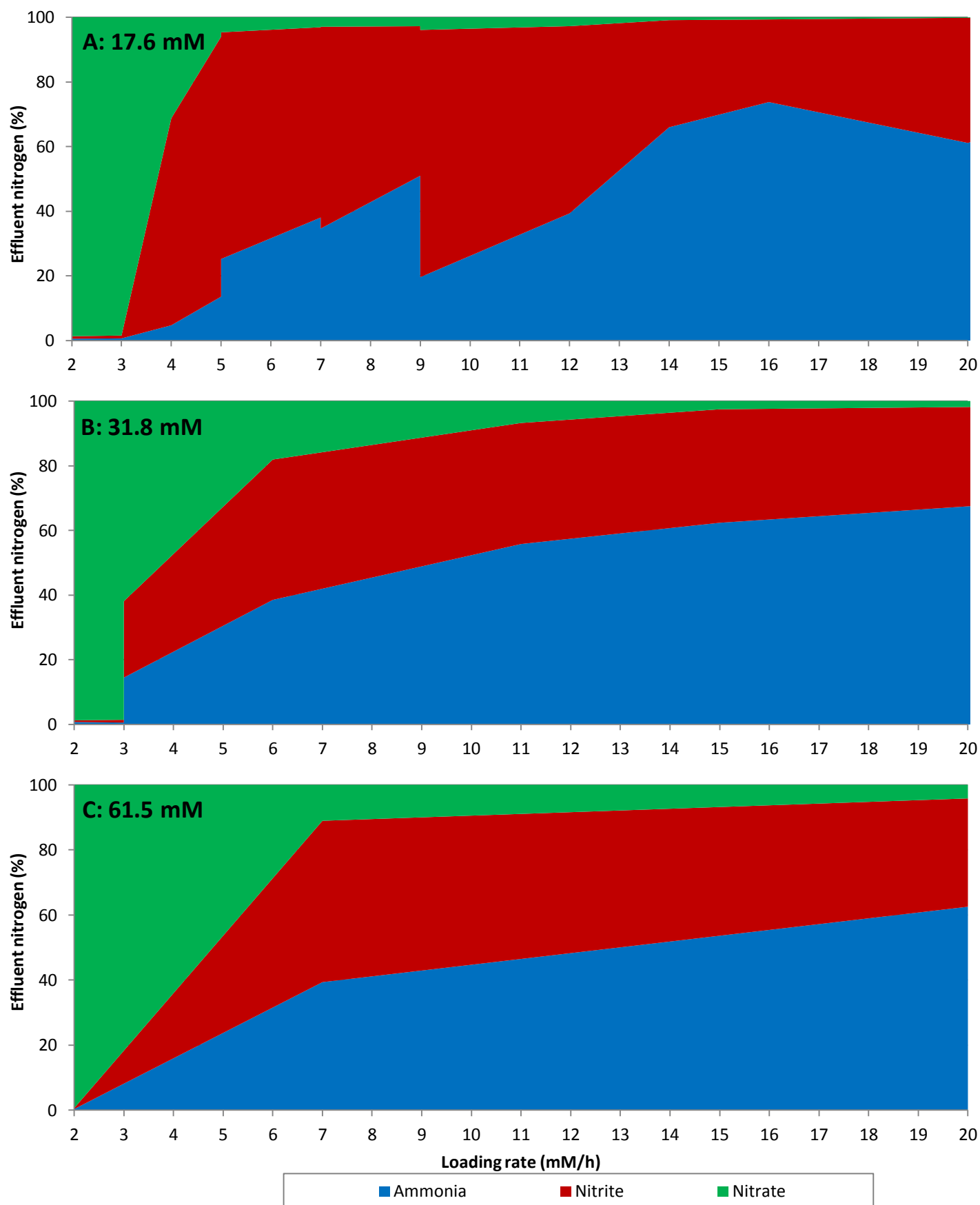


Figure 5.12 Composition of biofilm reactor effluent for A) 17.6 mM, B) 31.8 mM and C) 61.5 mM ammonia concentration.

5.5 Ammonia removal in batch MFC

5.5.1 Batch runs without mediator

Removal of ammonia (ammonia oxidation) was investigated in the anode compartment of the dual chamber microbial fuel cell, where a chemical electron acceptor, $\text{Fe}(\text{CN})_6\text{K}_3$, was used in the cathode. The MFC ammonia oxidation profile was very similar to the conventional reactor profiles in that ammonia has to be fully oxidized to nitrite before any nitrite oxidation to nitrate can take place. In the first MFC trial with ammonia as a substrate, a concentrated solution of ammonia was added to anode to achieve about 18 mM of ammonia. Microbial cells grown in shake flasks were centrifuged and injected into the anode which contained medium purged with nitrogen and ammonia. Sampling of anodic liquid after a period of 24 h showed a drastic decrease in ammonia concentration to 3 mM with no corresponding increase in nitrite or nitrate (Figure 5.13 A). Over the next 1000 hours the remaining ammonia concentration slowly fell to zero and nitrite concentration built up. The nitrite reached about 8 mM when all the ammonia was removed and after that the nitrite started to get oxidized to nitrate. The nitrate quickly built up to 18 mM which corresponds to the original molar amount of ammonia.

This indicated that the original decrease in ammonia observed after 24 h must have been the result of a very fast non-biological phenomenon occurring at the start of the ammonia oxidation. Subsequent trials showed that this phenomenon was due to the diffusion of ammonium ions into the cathode chamber. After the first trial, experiment with 18 mM ammonia was repeated under the same conditions. As seen in Figure 5.13 B, ammonia oxidation characteristic is exactly the same as the first trial. In this case a higher nitrite build up occurred where over a period of 1300 hours the nitrite concentration reached to a value around 11 mM. At this point, despite the presence of 1 mM residual ammonia in the MFC, ammonia oxidation did not continue and no nitrate was produced. This experiment was stopped due to time restraint. One could speculate that by giving sufficient time, oxidation of ammonia and nitrite oxidation might have proceeded to completion.

In the next experiment, a slightly higher ammonia concentration of 31 mM was used in the MFC. Similar to previous run, the ammonia concentration dropped to about 9 mM during the initial period of experiment, then over the next 800 hours the ammonia concentration further dropped to 2 mM and the nitrite concentration reached 16 mM (Figure 5.13 C). Following this, the increasing trend of nitrite concentration leveled off and ammonia, nitrite, and nitrate concentrations did not change anymore. To assess whether presence of nitrite at high concentration inhibited the microbial activity, at around 1500 h 50 mL of the liquid was removed from the reactor and was replaced with fresh medium with no ammonia. The residual concentrations of the nitrite and ammonia in the MFC were reduced to 9.5 and 1.5 mM as a result of this dilution. After the dilution of the reactor content, the nitrite production started up again and nitrite concentration increased to 14.5 mM. This seems to indicate that there was an inhibition of ammonia oxidation due to combined effects of nitrite and ammonia presence in the reactor. The reactor potential data were consistently between 300 to 400 mV for all the batches. The potential dropped to near zero when various resistors were placed between the anode and the cathode to complete the circuit. This was done in order to measure the current and the power generated by the fuel cell. The highest potentials are read during the first phase of the oxidation when there is very little nitrite build up, but as the nitrite concentration dramatically increases the potential starts to go down until nitrate production, then the potential stays the same.

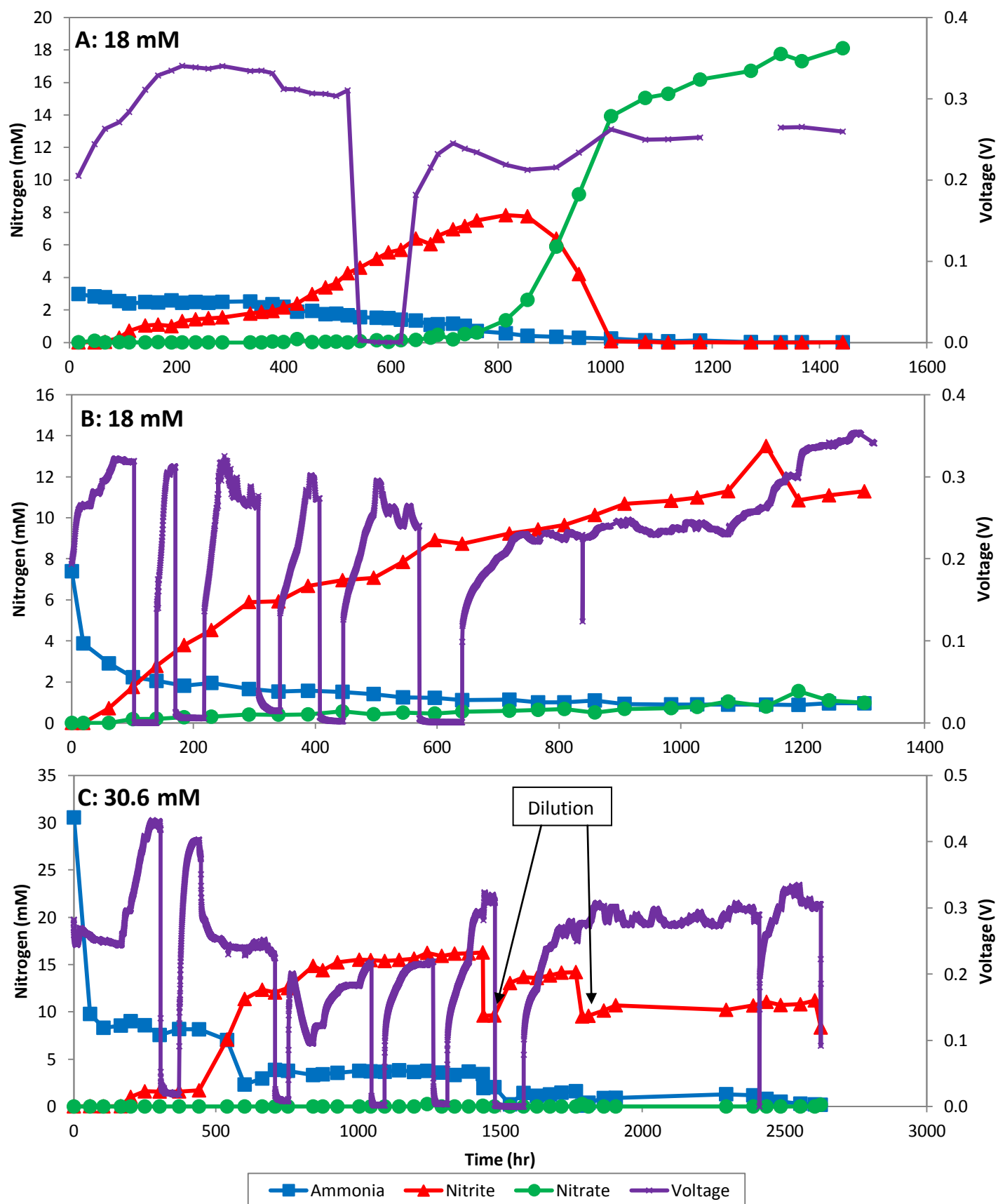


Figure 5.13 MFC ammonia oxidation with A) completed 18.1 mM, B) incomplete 18 mM, and C) incomplete 30.6 mM ammonia batch results.

The most challenging aspect of ammonia treatment was the reproducibility of the results as far as complete oxidation of ammonia to nitrate was concerned. Results showed that while it is possible to fully oxidize ammonia to nitrate under the anaerobic condition of the fuel cell, complete oxidation is sometimes difficult to reproduce. At this point it is not known why in some cases complete oxidation of ammonia to nitrate did not occur but the reasons might include variation in make-up of the culture, dominance of the ammonia oxidizing bacteria as opposed to nitrite oxidizing counterparts, and inhibition from byproducts, bicarbonate shortage, or catholyte depletion.

5.5.2 Batch runs with mediator

In order to see whether addition of an electron mediator could address the problem of reproducibility with ammonia oxidation in the microbial fuel cell, electron mediator resazurin was used in the next set of experiments. Resazurin concentration in the anodic medium was 1 mM which made the medium extremely dark blue. Over time as oxidation commenced in the anodic chamber, the medium turned purplish. The results of the first batch using electron mediator is seen in Figure 5.14. The most promising part of this result was the complete oxidation of ammonia to nitrate within 1000 hours. After this first trial, resazurin was used in all other MFC ammonia oxidation trials.

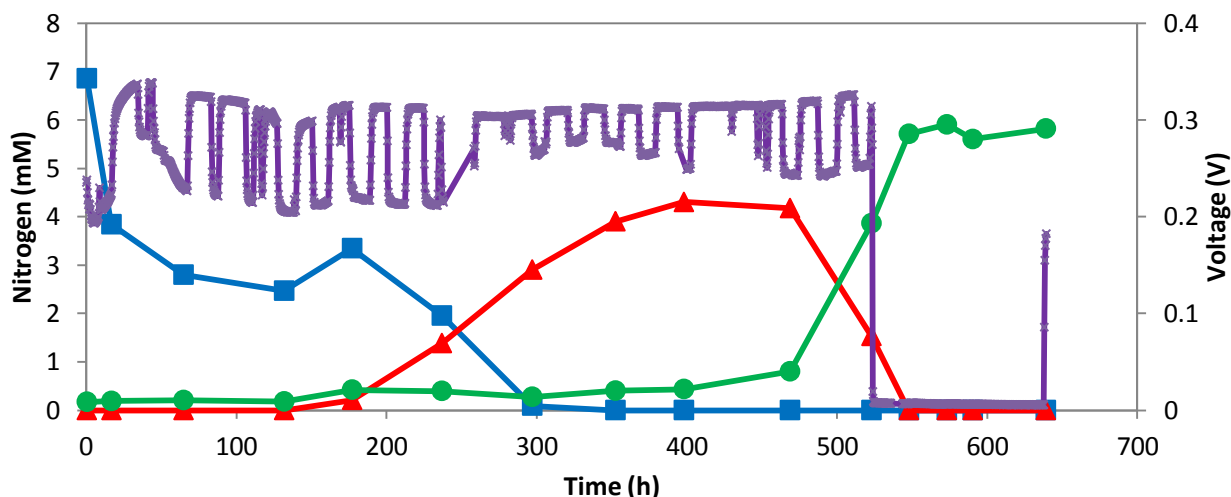


Figure 5.14 First ammonia oxidation batch with resazurin as electron mediator.

Figure 5.15 presents the data for ammonia concentration batches of 8 mM (Figure 5.15 A), 11 mM (Figure 5.15 B), and 24 mM (Figure 5.15 C) in the microbial fuel cell. The ammonia oxidation in the presence of mediator was much faster than that in the absence of mediator. After the first ammonia oxidation batch in Figure 5.14, the same cells were harvested and used for the subsequent trials whose results are seen in Figure 5.15. Complete oxidation of 8 mM ammonia to nitrate took under 100 hours, while complete oxidation of 11 mM and 24 mM ammonia took 200 and 300 hours, respectively.

In all the resazurin trials, the same non-biological drop in ammonia concentration occurred soon after the start of the trial. It seems that the oxidation characteristics of ammonia have not been affected by the addition of the electron mediator, only the oxidation rate seems to have been affected and that in all trials oxidation of ammonia to nitrite and consequently to nitrate occurred in a consistent manner. Moreover, the concentration of nitrite accumulated in the reactor was a lot lower than what would be expected from the initial ammonia concentration. However, final nitrate concentration correlated well with the initial concentration of ammonia. Resazurin was shown to be safe to use with nitrifying bacteria over repeated trials (i.e. no negative impact on microbial activity).

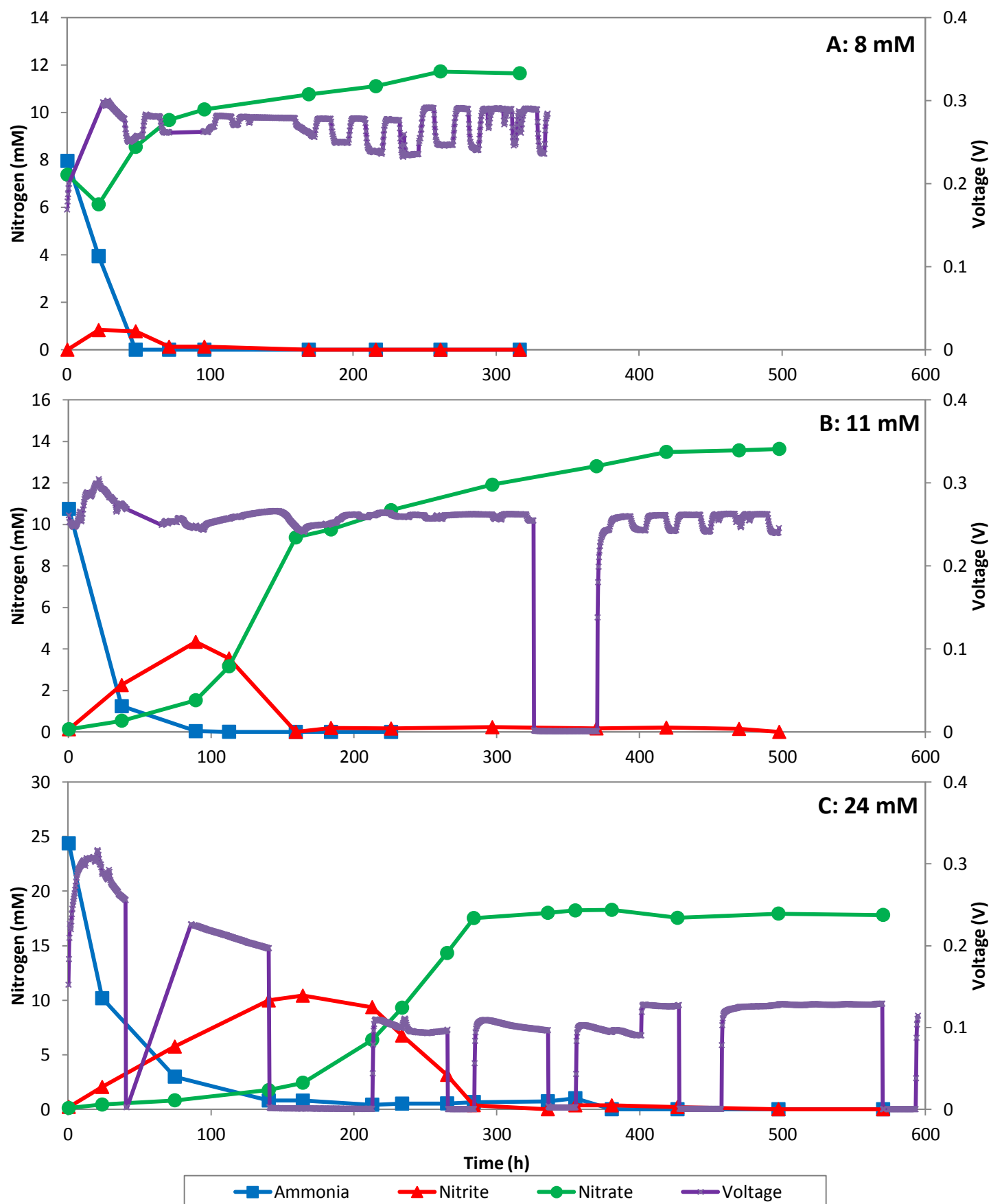


Figure 5.15 Ammonia oxidation in MFC with resazurin mediator containing A) 8 mM , B) 11 mM, and C) 24 mM ammonia.

5.6 Nitrite removal in batch MFC

When nitrite was used as the sole electron donor in the MFC anode chamber (nitritation), the results were promising and reproducible. Figure 5.16 shows the data for three consecutive runs with 15 mM of nitrite as substrate. Cell pellets from the cultures maintained on shake flasks (with nitrite as substrate) was used as the inoculum and concentrated sodium nitrite was injected to achieve the desired nitrite concentration. After the initial amount of nitrite was oxidized to nitrate (Figure 5.16A), the same anode was supplied with another 15 mM of nitrite (Figure 5.16B), and once the nitrite was oxidized the second time another 15 mM of nitrite was added (Figure 5.16C). Due to the lag phase, first sequence (Figure 5.16 A) lasted over 500 hours. The lag phase is likely the period required by the aerobic nitrifying bacteria to get acclimated to the anaerobic environment. After nitrite was fully oxidized at around 470 hours, the second sequence started immediately (Figure 5.16 B). The third oxidation was immediate again with no inhibition from over 30 mM of residual nitrate in the anode (Figure 5.16 C). All the nitrite was oxidized to nitrate under 100 hours in the second and third sequence, which is comparable to the nitrite oxidation in conventional reactors that took around 80 hours for the same concentration.

The open circuit voltages of the batches were around 300 mV for all three trials but dropped to lower values as loads (different resistors) were applied. While it is possible to improve the performance of the nitritation with sequential runs, the system potential does not seem to improve with repeated trials so other approaches may need to be used to improve the performance of the system (i.e. use of mediators that help transport the electrons to the anode or use of other MFC configurations).

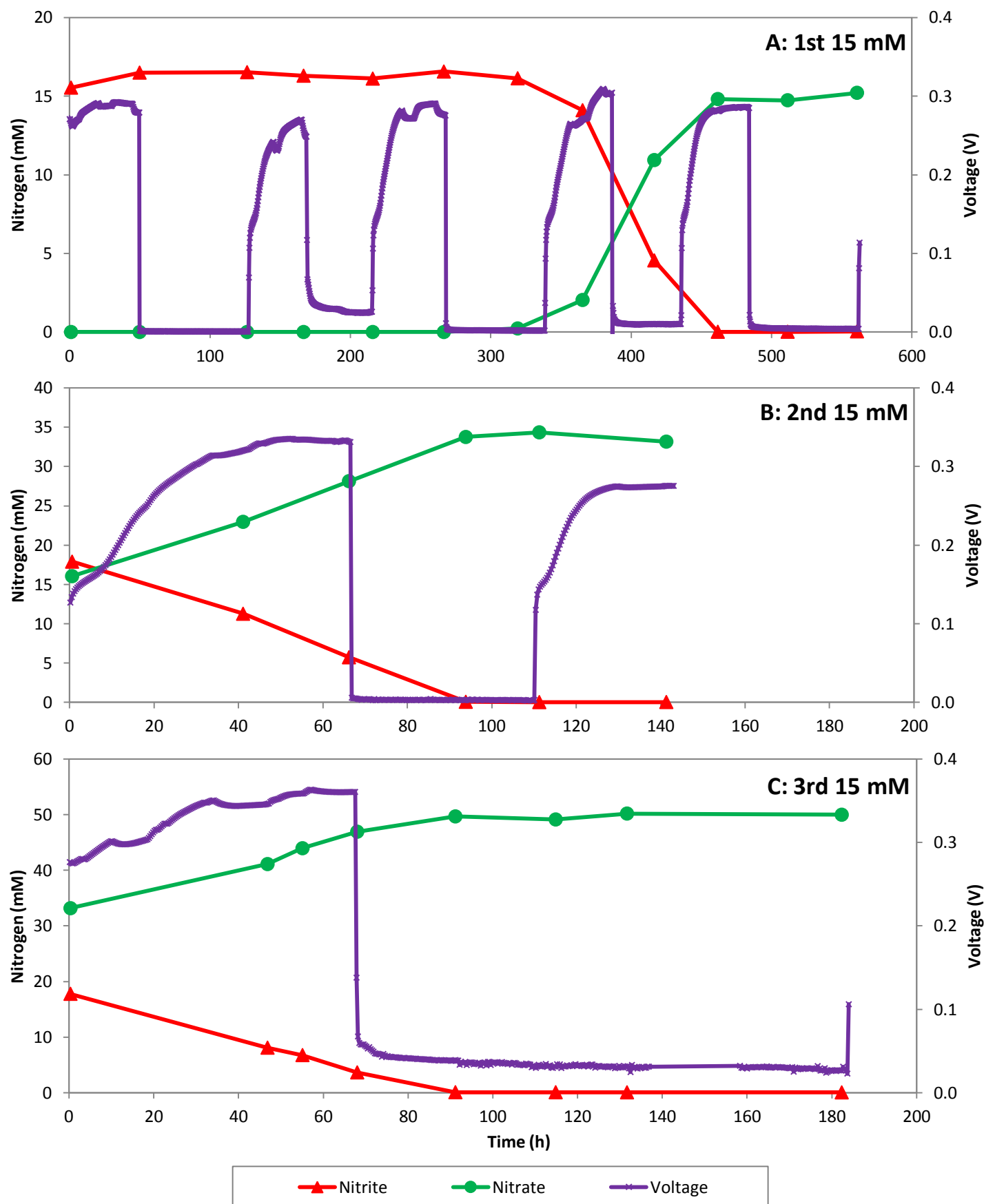


Figure 5.16 Sequential oxidation of nitrite: A) first batch, B) second batch, and C) third batch.

Similar sequential batch trials were carried out with 8 mM (Figure 5.17A), 35 mM (Figure 5.17C), and 60 mM (Figure 5.17D) nitrite in addition to the 18 mM (Figure 5.17B) trials. The data from these sequential trials showed the same trend, the first trial was the longest because of the lag phase at the beginning when the culture is acclimating to its environment, with no lag phase in the subsequent runs which took about the same amount of time as in the conventional batch reactors. Figure 5.17 data shows the results of the sequential trials after the lag phase has passed so either the second or third sequence results. These trials clearly identify a method of improving the performance of the MFC reactor by simply giving the microbial culture some time to adjust to the anodic conditions.

The 8 mM batch took about 40 hours to oxidize (Figure 5.17A), the 18 mM batch took about 100 hours (Figure 5.17B), the 35 mM took about 300 hours, and the 60 mM batch took about 700 hours to completely oxidize. The average time for nitrite oxidation in a conventional reactor for concentrations between 7 to 15 mM is about 80 hours, for 35 mM batch is about 100 hours, and for 60 mM batch is about 140 hours. So the lower concentration batches in MFC took about the same amount of time to oxidize all the nitrite as a conventional reactor. While higher concentrations such as 35 mM and 60 mM batches took longer time in the MFC than in the conventional reactor. The 35 mM batch took 200 hours longer and 60 mM batch took almost 600 hours longer in the MFC. This highlights the potential to shorten the treatment time in the MFC over repeated runs in the same reactor or through the use of electron mediator. The open circuit voltage reached up to 400 mV during nitrite oxidation and dropped to 300 mV when all the nitrite was oxidized to nitrate (Figure 5.17D).

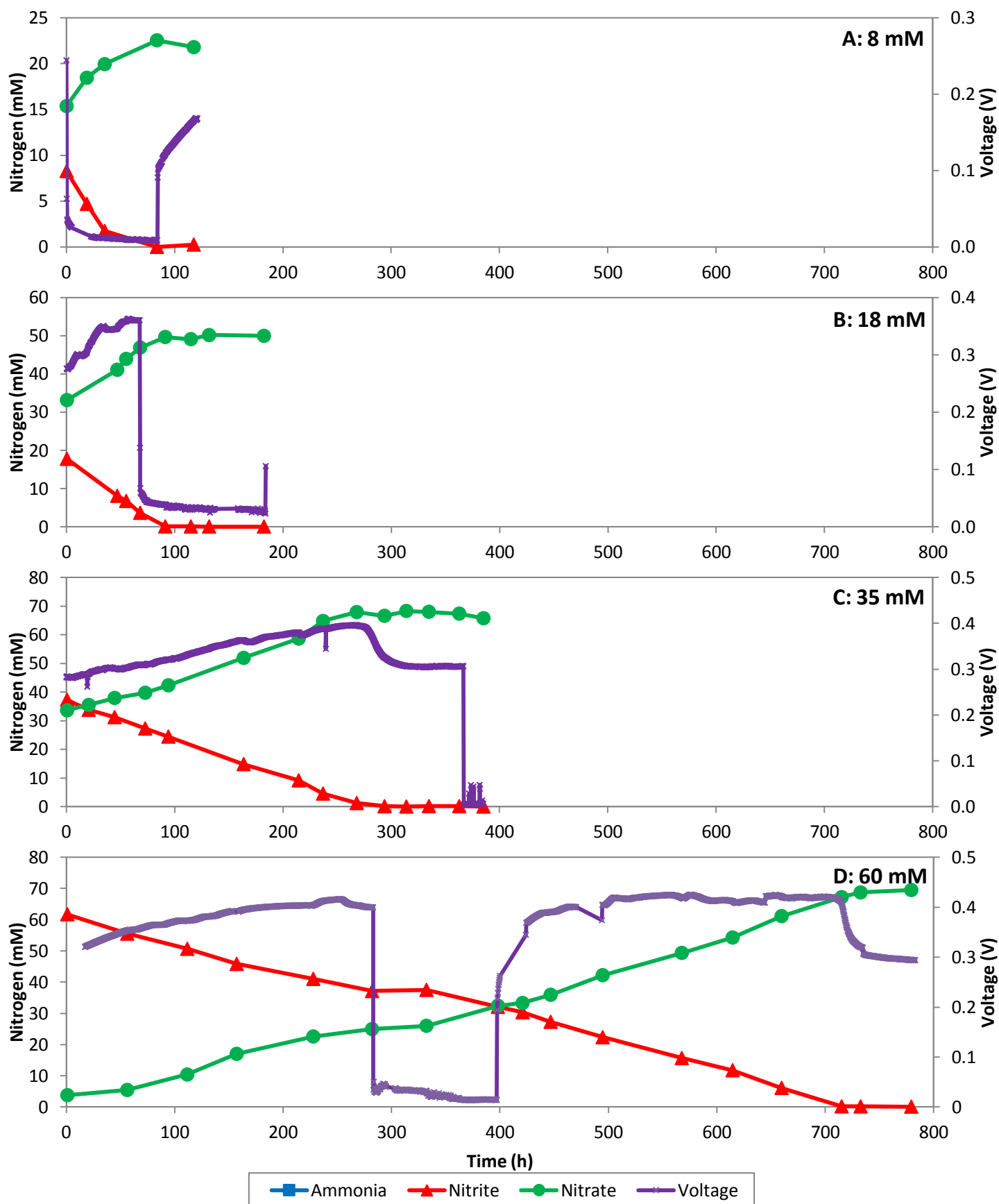


Figure 5.17 The nitrite oxidation batches without mediator at A) 8 mM, B) 18 mM, C) 35 mM, and D) 60 mM concentrations

5.7 Power density and current density preliminary analysis

The power density and the current density data was calculated for the ammonia oxidation and nitrite oxidation batch runs. Table 5.1 contains the current density and power density data for the ammonia and nitrite batches. The average current density and power density values for ammonia was 1.2 mA/m^2 and 0.013 mW/m^2 , respectively. The corresponding values for nitrite oxidation were 2.92 mA/m^2 and 0.022 mW/m^2 . The power densities achieved in other microbial fuel cell studies using substrates such as glucose, lactose, and acetate range from 0.00032 to 3600 mW/m^2 (Rabaey & Verstraete, 2005), while current densities for substrates such as phenol, xylose, and wastewater are in the range 0.004 to 2.05 mA/m^2 (Pant et al., 2010). While the current density values obtained in this experiment are in line with the literature values, the power density values are on the lower end of the scale. However, the vast majority of the literature values are obtained from oxidation of different forms of organic carbon and none of the studies cited in the Pant et al. (2010) and Rabaey and Verstraete (2005) reviews used ammonia or nitrite as sole substrate. In addition, many of these other studies used a more complex microbial fuel cell and anode designs. While this study used the simple MFC design with large internal resistances and anode with small surface area.

Table 5.1 Current and power density data for ammonia and nitrite oxidation reactions in MFC.

Substrate	Current density (mA/m^2)			Power density (mW/m^2)		
	Max.	Ave.	St. Dev.	Max.	Ave.	St. Dev.
ammonia	4.37	1.20	1.06	0.181	0.013	0.041
nitrite	8.17	2.92	1.75	0.095	0.022	0.028

6 SUMMARY OF FINDINGS

The biological nitrification reactions takes place sequentially where the ammonia is completely oxidized to nitrite before the oxidation of nitrite to nitrate proceeds. This is true even though nitrite oxidation rate is much faster than that of ammonia oxidation and that prior to complete exhaustion of ammonia, nitrite has accumulated in the system at high concentrations. The batch results, both for ammonia and nitrite as substrate, show that increase of the initial substrate concentration led to a linear increase of oxidation rate. For similar initial concentrations, the rate of nitrite oxidation was about three times faster than that of ammonia, while oxidation rate of biologically produced nitrite (resulting from oxidation of ammonia) was about twice as fast as ammonia oxidation.

Two different reactor configurations (CSTR with free cells and Biofilm) used for continuous oxidation of ammonia displayed major differences when it came to ammonia removal capabilities. The biofilm reactor consistently outperformed the CSTR on the basis of sustaining higher maximum ammonia loading rate (27 mM/h as opposed to 8 mM/h) and maximum ammonia removal rate (9 mM/h as opposed to 3.5 mM/h). The dependency of ammonia removal rate on its loading rate showed saturation characteristics where the removal rate reached the maximum and remained relatively constant with further increase of loading rate for the range of rates tested in this work. In both CSTR and the biofilm reactors, effluent composition (ammonia, nitrite, and nitrate concentrations) can be controlled through the ammonia loading rate (residence time). The feed concentration made little difference on the effluent composition in the biofilm reactor, while the nitrite portion in the CSTR decreased from about 60 to 20% and ammonia increased from 40 to 70% as feed concentration was increased from 17.6 mM to 63.2 mM.

Although ammonia oxidation in the batch MFC took much longer than that in a conventional batch reactor, results of the present study clearly proved for the first time the possibility of biological oxidation of ammonia in MFC evident from formation of nitrite and nitrate. Contrary to previously

reported data, physicochemical processes are not the main phenomena causing the removal of ammonia in a microbial fuel cell. Oxidation of ammonia in MFC reactor appeared challenging as far as reproducibility was concerned. There was an instantaneous diffusion of the majority of ammonia in the anode into the cathode. However, this does not seem to be irreversible or permanent since at the end of the batch the final nitrate concentration corresponded with the starting ammonia concentration. After complete oxidation of ammonia to nitrite, it was relatively easy for the nitrite to be converted to nitrate. When resazurin was used as an electron mediator in the ammonia oxidation batches, the oxidation rate was much faster though the nitrification characteristics (oxidation of ammonia to nitrite and consequently to nitrate) did not change.

Nitrite oxidation in the MFC was much more consistent and reproducible. The nitrite removal was a result of a biological reaction, as produced nitrate correlated well with the amount of oxidized nitrite. In sequential batch runs, the performance of the MFC improved after each consecutive trial, to the point where the oxidation rate was comparable to that in an aerated conventional reactor. This highlights the importance of maintaining the microbial population in the MFC and repeated use of cells. This can be achieved using biofilm support materials in the anode; however the ability of the bacteria to transfer electrons in such system needs further investigation.

6.1 Recommendation for future work

Since continued acclimation of bacteria in the MFC environment seems to greatly improve the oxidation rate of ammonia and nitrite, using microbial cell in the biofilm state which allows for easy retention and repeated use of the acclimated bacteria is recommended and should be investigated. As part of this investigation various matrices with high porosity and surface area which also have the ability to serve as the electrode (i.e. graphite felt or sheet) should be investigated.

Considering the continuous nature of the wastewater treatment systems, performance of the MFC in removal of ammonia in continuous mode needs to be studied. Ammonia concentration and loading rate are the important parameters which would affect the performance of the system and their effects require a detailed evaluation. Following the development of a biocathode (specially a denitrifying biocathode) which is currently being investigated in our lab, potential for coupling of nitrification (anodic process) with denitrification (cathodic process) and development of a complete MFC needs to be investigated. Finally, the MFC configuration used in this research is suitable to study the biological processes involved in removal of ammonia through nitrification but not the most suitable system as far as generation of electricity is concerned, thus future works should also study the process of nitrification in other MFC configurations such as stacked and single chamber MFCs.

Bibliography

- Ahn, Y.-H., 2006. Sustainable nitrogen elimination biotechnologies: A review. *Process Biochemistry*, 41(8), pp.1709-21.
- Anthonisen, A.C., Loehr, R.C., Prakasam, T. & Srinath, E.G., 1976. Inhibition of nitrification by ammonia and nitrous acid. *Journal Water Pollutant Control Federation*, 48(5), pp.835-52.
- Art, H.W., 1993. *A dictionary of ecology and environmental science*. New York: Henry Holt and Company.
- Bartholomew, C.H., 2001. Mechanisms of catalyst deactivation. *Applied Catalysis A: General*, 212(1-2), pp.17-60.
- Cheng, S., Liu, H. & Logan, B.E., 2006. Increased performance of single chamber microbial fuel cells using an improved cathode structure. *Electrochemistry Communications*, 8(3), pp.489-94.
- Cheng, S., Liu, H. & Logan, B.E., 2006. Power density using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. *Environmental Science and Technology*, 40(1), pp.364-69.
- Clauwaert, P. et al., 2007. Biological denitrification in microbial fuel cell. *Environmental Science & Technology*, 41(9), p.3354–3360.
- Cuidad, G. et al., 2005. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. *Process Biochemistry*, 40(5), pp.1715-19.
- Du, Z., Li, H. & Gu, T., 2007. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotechnology Advances*, 25(5), pp.464-82.
- Gleick, P.H. et al., 2009. *The World's Water 2008-2009*. Washington, DC: Island Press.
- GRACE Communications Foundation, 2011. *Issues: Water Pollution*. [Online] Available at: <http://www.sustainabletable.org/issues/waterpollution/> [Accessed 3 January 2011].
- He, Z. & Angenent, L.T., 2006. Application of bacterial biocathodes in microbial fuel cells. *Electroanalysis*, 18(19-20), pp.2009-15.
- He, Z. et al., 2009. Electricity production coupled to ammonium in a microbial fuel cell. *Environmental Science and Technology*, 43(9), pp.3391-97.
- Kim, J.S. et al., 2005. Effects of temperature, oxidant and catalyst loading on the performance of direct formic acid fuel cell. *Korean Journal of Chemical Engineering*, 22(5), pp.661-65.
- Kim, J.R., Zuo, Y., Regan, J.M. & Logan, B.E., 2008. Analysis of ammonia loss mechanisms in microbial fuel cells treating animal wastewater. *Biotechnology and Bioengineering*, 99(5), pp.1120-27.
- Liu, Z., Liu, J., Zhang, S. & Su, Z., 2008. A novel configuration of microbial fuel cell stack bridged internally through an extra cation exchange membrane. *Biotechnology Letters*, 30(6), pp.1017-23.

- Liu, H., Ramnarayanan, R. & Logan, B.E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environmental Science and Technology*, 38(7), pp.2281-85.
- Lu, N. et al., 2009. Electricity generation from starch processing wastewater using microbial fuel cell technology. *Biochemical Engineering Journal*, 43(3), pp.246-51.
- Milne, G.W.A., 2005. *Gardner's Commercially Important Chemicals*. Hoboken, New Jersey: Wiley & Sons, Inc.
- Min, B. et al., 2005. Electricity generation from swine wastewater using microbial fuel cells. *Water Research*, 38(20), pp.4961-68.
- Morris, J.M., Fallgren, P.H. & Jin, S., 2009. Enhanced denitrification through microbial and steel fuel-cell generated electron transport. *Chemical Engineering Journal*, 153(1-3), pp.37-42.
- Morris, J.M. & Jin, S., 2009. Influence of NO₃ and SO₄ on power generation from microbial fuel cells. *Chemical Engineering Journal*, 153(1-3), pp.127-30.
- Nolan, B.T., Hitt, K.J. & Ruddy, B.C., 2002. Probability of nitrate contamination of recently recharged groundwaters in the conterminous United States. *Environmental Science and Technology*, 36(10), pp.2138-45.
- Nowak, E. et al., 2008. Characterization of formulated microbial products by denaturing gradient gel electrophoresis, total cellular fatty acid analysis, and DNA microarray analysis. *Canadian Journal of Microbiology*, 54(5), pp.380-90.
- O'Brien, J., Wilson, I., Orton, T. & Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *European Journal of Biochemistry*, 267(17), pp.5421-26.
- Oh, S., Min, B. & Logan, B.E., 2004. Cathode performance as a factor in electricity generation in microbial fuel cells. *Environmental Science and Technology*, 38(18), pp.4900-04.
- Pant, D., van Bogaert, G., Diels, L. & Vanbroekhoven, K., 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresource Technology*, 101(6), pp.1533-43.
- Paredes, D. et al., 2007. New aspects of microbial nitrogen transformations in the context of wastewater treatment - A review. *Engineering Life Science*, 7(1), pp.13-25.
- Park, H.I., Kim, D.k., Choi, Y.-J. & Pak, D., 2005. Nitrate reduction using an electrode as direct electron donor in a biofilm-electrode reactor. *Process Biochemistry*, 40(10), pp.3383-3388.
- Rabaey, K. & Verstraete, W., 2005. Microbial fuel cells: novel biotechnology for energy generation. *Trends in Biotechnology*, 23(6), pp.291-98.

- Rozendal, R.A. et al., 2008. Towards practical implementation of bioelectrochemical wastewater treatment. *Trends in Biotechnology*, 26(8), pp.450-59.
- Shuler, M. & Kargi, F., 2006. *Bioprocess Engineering: Basic Concepts*. 2nd ed. Prentice Hall.
- Siripong, S. & Rittmann, B.E., 2007. Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. *Waste Research*, 41(5), pp.1110-20.
- Strous, M., Heijnen, J.J. & Kuenen, J.G., 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology*, 50(5), pp.589-96.
- Sukkasem, C. et al., 2008. Effect of nitrate on the performance of single chamber air cathode microbial fuel cells. *Water Research*, 42(19), pp.4743-50.
- Sund, C.J. et al., 2007. Effect of electron mediators on current generation and fermentation in a microbial fuel cell. *Applied Microbial Biotechnology*, 76(3), pp.561-68.
- Suthersand, S. & Ganczarczyk, J.J., 1986. Inhibition of nitrite oxidation during nitrification. *Water Pollution Research Journal Canada*, 21(2), pp.257-66.
- Tijhuis, L., van Loosdrecht, M.C.M. & Heijnen, J.J., 1995. Formation of nitrifying biofilms on small suspended particles in airlift reactors. *Biotechnology and Bioengineering*, 47(5), pp.585-95.
- U.S. Environmental Protection Agency, 2000. *National Water Quality Inventory: Report 2000*. U.S. EPA.
- Vadivelu, V., Yuan, Z., Fux, C. & Keller, J., 2005. The inhibitory effects of nitrite on the energy generation and growth processes of an enriched *Nitrobacter* culture. In *4th IWA Activated Sludge Population Dynamics Specialist Conference*, 2005.
- van der Star, W.R.L. et al., 2007. Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research*, 41(18), pp.4149-63.
- van Kempen, R., Mulder, J.W., Uijterlinde, C.A. & Loosdrecht, M.C.M., 2001. Overview: full scale experience of the SHARON process for treatment of rejection water of digested sludge dewatering. *Water Science and Technology*, 44(1), pp.145-52.
- Viridis, B. et al., 2010. Simultaneous nitrification, denitrification and carbon removal in microbial fuel cells. *Water Research*, 44(9), pp.2970-80.
- Viridis, B., Rabaey, K., Yuan, Z. & Keller, J., 2008. Microbial fuel cells for simultaneous carbon and nitrogen removal. *Water Research*, 42(12), pp.3013-24.
- Wiesmann, U., 1994. Biological nitrogen removal from wastewater. In Fletcher, A. *Advances in Biochemical Engineering Biotechnology*. Berlin: Springer-Verlag. pp.113-54.

Wilkinson, S., Klar, J. & Applegarth, S., 2006. Optimizing Biofuel Cell Performance Using a Targeted Mixed Mediator Combination. *Electroanalysis*, 18(19-20), pp.2001-07.

World Health Organization, 2011. *Water Sanitation and Health*. [Online] Available at: http://www.who.int/water_sanitation_health/diseases/methaemoglob/en/ [Accessed 4 January 2011].

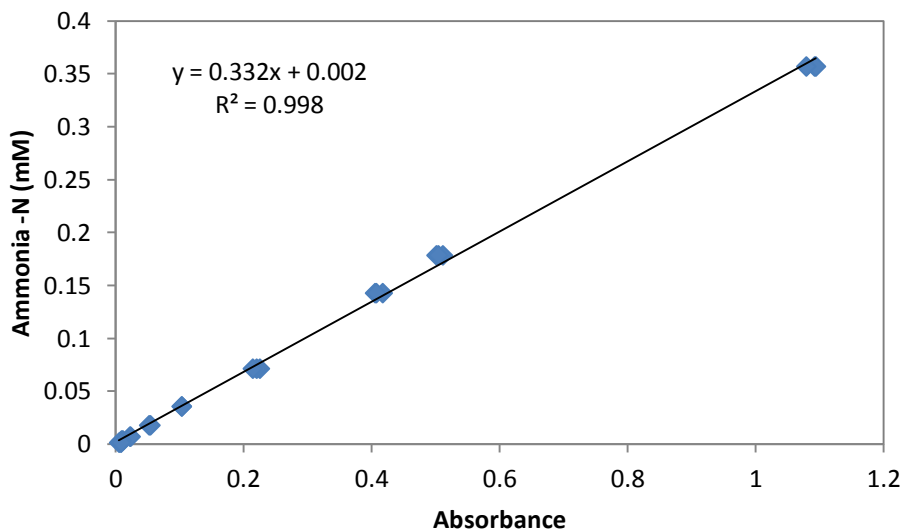
World Water Assessment Programme, U.N. & UNESCO, 2003. *Water for People, Water for Life: The United Nations World Water Development Report*. Barcelona: Berghahn Books.

You, S.-J. et al., 2009. Improving phosphate buffer-free cathode performance of microbial fuel cell based on biological nitrification. *Biosensors & bioelectronics*, 24(12), pp.3698-701.

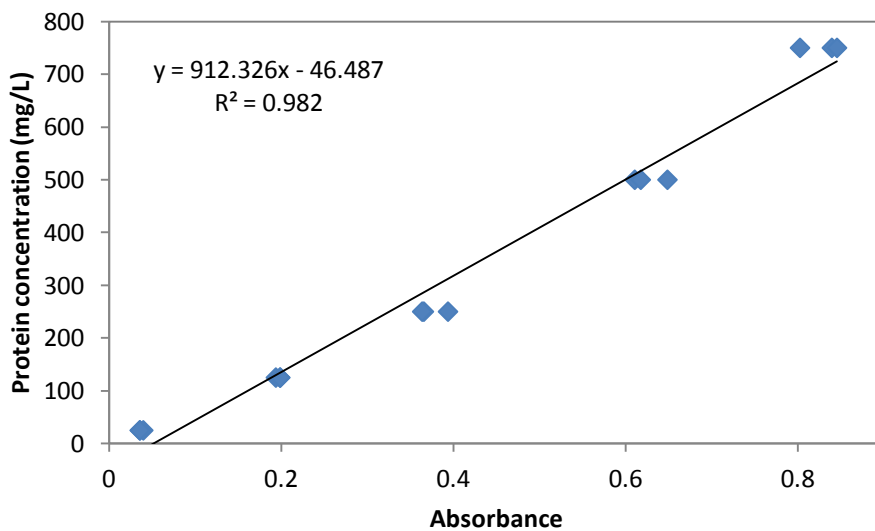
Zhu, G. et al., 2008. Biological removal of nitrogen from wastewater. *Reviews of environmental contamination and toxicology*, 192, pp.159-95.

Appendix A

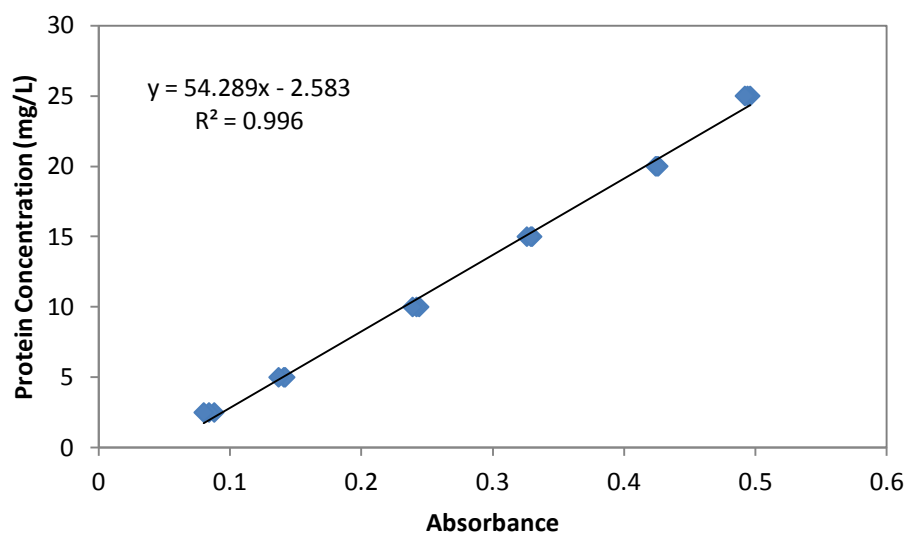
Standard Curves



Ammonium nitrogen concentration standard curve determined with Nessler reagent measured using spectrophotometer at 425 nm wavelength.



Protein concentration standard curve determined with Coomassie assay measured using spectrophotometer at 595 nm wavelength. For 50 to 700 ppm range.



Protein concentration standard curve determined with Coomassie assay measured using spectrophotometer at 595 nm wavelength. For 0 to 50 ppm range.